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ORIGIN AND DEVELOPMENT OF THE PYCNIDIUM

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(WITH PLATES XVII–XXII)

Introduction

The Ascomycetes have been the subject of repeated investigations, especially in regard to the morphology of the perithecium and the cytology and sexuality of the ascigerous stage. The conidial stages, placed in the Fungi Imperfecti in the absence of knowledge of their ascigerous stages, of necessity are classified on a basis of the morphology of the conidia-bearing structures. For this reason, and because the pycnidia are said sometimes to develop directly into perithecia, extension of our knowledge of the morphology of such conidiiferous structures as the pycnidium and the acervulus is highly desirable.

The references in literature to the development of the pycnidium are, for the most part, merely incidental, and studies directly devoted to the subject are few. As early as 1876 BAUKE (5) described what he considered as 3 methods of pycnidial development, without giving names to them. In 1884, however, DEBARY (9) distinguished 2 main methods of development, which he designated as "symphogenous" and "meristogenous": "symphogenous" when the pycnidial primordium arises through the interweaving of young hyphal branches to form a network that is at first loosely woven, but later becomes compact and knotlike;

"meristogenous" when the primordium arises from a single hyphal cell or a group of adjacent cells of a single hypha by continued cross and longitudinal division. In the latter, branches from neighboring cells or from the mass itself may share. A number of variations of these 2 methods have been described. Classification of the pycnidial developments described by BAUKE according to the methods distinguished by DEBARY places *Cucurbitaria* as meristogenous, *Diplodia* as symphogenous, and *Pleospora polytricha* as a combination of the two.

ZOPF (**52**) in 1890 mentioned 3 types of pycnidial development: "Hyphenfrucht," "Gewebefrucht," and "Knäuelfrucht." The "Hyphenfrucht" comprises those in which development proceeds from a single hyphal cell which by dividing and swelling forms a quadrant, from which with the aid of 2 or 3 neighboring cells a primordium is formed. By "Gewebefrucht" is designated those instances in which a tissue mass is formed. This mass may develop in either of 2 ways: by neighboring cells of a hypha becoming septate, swelling, dividing, becoming more rounded, while short dividing hyphae either from the mass or from neighboring cells of the hypha share in the formation; or in a like manner it may be formed from cells of 2 or more contiguous hyphae. The "Knäuelfrucht" develops a primordium from 1 or more short hyphal branches which coil spirally, branch, and interweave into a knot which later develops into the pycnidium. The "Hyphenfrucht" and "Gewebefrucht" are clearly variations of the meristogenous method of development of DEBARY. The other, "Knäuelfrucht," is symphogenous.

In addition to these should be mentioned the "sporopycnidium" of VON TAVEL (**46**), referred to also by PLANCHON (**29**) and SCHNEGG (**33**). This pycnidium arises meristogenously, according to VON TAVEL, representing the most extreme case of meristogenous development. A single spore in the presence of abundant food germinates and grows a short mycelium; then by division and growth becomes a pycnidium. REDDICK (**31**) notes that pycnosclerotia may be found in *Guignardia*. These bodies arise as pycnidia, produce pycnidiospores, and later function as perithecia, producing asci and ascospores.

Although in many instances the fungi were not studied primarily as to their pycnidial development, and such information as was collected regarding this was incidental, according to the figures and descriptions given the following belong to the meristogenous group of DEBARY: *Pleospora* (GIBELLI and GRIFFINI 17; BAUKE 5), *Cucurbitaria* (EIDAM 14; BAUKE 5), *Pycnis* (BREFELD 6), *Fumago* (TULASNE 43; ZOPF 53; SCHOSTAKOWITSCH 34), *Sphaeronaema* (HALSTED and FAIRCHILD 18), pycnidium with *Teichospora* (NICHOLS 27), *Phyllosticta* with *Alternaria* (PLANCHON 29), *Sphaeropsis* and *Coniothyrium* (POTEBNIA 30), *Chaetophoma* (ARNAUD 3), *Phoma* (REDDICK 31; MERCER 26; SCHNEGG 33), *Endothia* (ANDERSON 1). As symphogenous may be classed: *Cicinnobolus* (DEBARY 8), *Fumago* (TULASNE 43; ZOPF 52), *Diplodia* (BAUKE 5; VAN DER BIJL 44), *Graphiola* (FISCHER 15), *Cystispora* (VON TAVEL 46), *Hendersonia* (VOGES 45; WOLF 51), pycnidium with *Sphaerella* (HIGGINS 21), *Sphaeropsis* (HESLER 19), *Septoria* (LEVIN 25). A few of the species have both types of development.

The present investigation is an endeavor to extend our knowledge of the very early stages in the development of the pycnidium and kindred structures.

Methods

For the present study, the fungi were either isolated from their hosts or cultures were procured from various sources as noted. Corn meal agar (35) was used because it gave an abundant growth, and its transparency facilitated the study of even the youngest stages. Other media were used in a few instances for comparison. Cultures for study were grown in Petri dishes, at room temperature (15–37° C.). Transfers were made by lifting hyphae or spores from pure cultures to the poured agar in Petri dishes. Drop cultures of melted agar were made by the use of sterile pipettes. In some cases the drop of agar was inoculated with spores as for dilution plating. In others, each drop was inoculated by transfer. These drop cultures served well for study of early stages of development, but seldom produced mature conidiiferous structures.

Disks of agar containing young pycnidia were removed from Petri dish cultures, placed on cover glasses, and inverted on

Van Tieghem rings in order to follow further the development of the pycnidia. Growth in Petri dishes was observed periodically, the intervals depending upon the rate of growth of the organism. Pycnidia of each species were kept under observation during their entire period of early development. For close detailed study and for drawing, mounts were made by removing from Petri dish cultures small squares of agar containing pycnidia in various stages of development, mounting them either in water for immediate study, or in glycerine for later study. Material from test tube cultures was teased out and studied for comparison and verification of many points.

Following the usage of earlier writers, DEBARY (9), BAUKE (5), ZOPF (52), and VON TAVEL (46), the word primordium is used throughout this paper to designate a group of cells that have become so differentiated that it is clearly evident that from them a pycnidial or similar structure will arise.

Genera and species studied

PHOMA (Fries) Desmazières

Phoma herbarum West; isolated from its host, *Polygonum hydropiper* L., at Urbana, Illinois, July 8, 1916.

Pycnidia are produced in cultures in moderate abundance. All stages of development, from the mature pycnidia on older mycelium to beginning stages on the younger mycelium, can be observed even in a small sector of a Petri dish culture. Development proceeds typically from a single cell of a hypha. This cell divides both transversely and diagonally (fig. 1) into a few cells which swell and enlarge. These cells by continued swelling and dividing form an irregular mass with very few or no hyphal branches. This mass shows distinctly its origin from a single hypha (figs. 1–3). As the mass continues to enlarge, one side protrudes slightly as a short rostrum which becomes lighter in color (fig. 4). This is the primordium which later becomes a pear-shaped pycnidium with an ostiole from which spores are discharged (fig. 5). The development is a typically simple meristogenous one.

Phoma pirina (Fries) Cooke; isolated at Urbana, Illinois, May 10, 1916, from dead twigs of *Pyrus communis* L. from Savoy, Illinois.

This fungus produces abundant small pycnidia 60–120 μ in diameter, at first closed but later with an ostiole 20–30 μ wide. The spores vary in size from 5×3 to 10×4 μ. A few cells of the hypha swell, divide both crosswise and diagonally, swell, and divide again (figs. 6, 7). This small mass continues to enlarge by cell division, and a few hyphal branches bud out from it (fig. 8). By continued development an irregular mass (fig. 9) is formed, which is the primordium of the pycnidium. In a few cases closely lying hyphae may take part in the formation (figs. 10–13), but the typical method of development is the simple meristogenous one from a few cells of a single hypha.

Phoma destructiva Plowr.; isolated at Urbana from fruits of tomato (*Lycopersicon esculentum* Mill.) in September 1915. In all respects the disease and the fungus agreed with the one described by Miss JAMIESON (**22**).

The pycnidial primordium usually arises meristogenously. In most cases a single hypha gives rise to the primordium (figs. 14–17); in other cases 2 or more hyphae share in its formation (figs. 18, 19, 22). In either case adjacent cells swell and divide crosswise and diagonally until a rounded or elongated mass is formed, slightly darker in color than the mycelium. The former (fig. 15) is the simple type of meristogenous development.

Phoma, species indet.; isolated from stems of clover (*Trifolium pratense* L.) at Urbana, Illinois, in July 1916.

Development takes place in a simple meristogenous manner. One hypha only is involved in the formation of the primordium (figs. 23–27). Short hyphal threads sometimes branch from the mass. The cells are usually large and swell into a rounded shape. The mass develops more rapidly at one end than at the other, so that the young pycnidium is slightly conical.

Phoma, species indet.; isolated from a berry of the Concord variety of grape (*Vitis labrusca* L.), from a vineyard near Collinsville, Illinois, September 1917.

A portion of the culture seen under the high power of the microscope showed a very characteristic arrangement of hyphae and of

pycnidia in various stages of development (figs. 28–33). The simple meristogenous development of the primordium is similar to that described for *Phoma herbarum* West (figs. 1–5).

Phoma cichorii Passr.; isolated from *Phlox divaricata* L. by Mrs. ESTHER YOUNG TRUE, at Urbana, Illinois, November 1917.

A few adjacent cells of a single hypha or of a number of contiguous hyphal strands divide into short cells (figs. 34, 35). By budding and swelling, short hyphal branches arise, the cells of which swell and divide (fig. 36). The cells of the mother strand or strands also enlarge and divide. The component hyphae anastomose, forming a pseudoparenchymatous, irregularly rounded mass. This mass develops into the pycnidium. The development of this fungus differs from that described for the other species in that, where only a single strand is involved, the mode is simple meristogenous with the original cells and their budding branches anastomosing to form the mass (fig. 36). In instances where 2 or more strands are involved (fig. 37), there has been a similar division, swelling, and branching in each strand, but the whole has been united into a single primordium as described for *Phoma destructiva* (fig. 22). This mode is compound meristogenous.

MACROPHOMA (Sacc.) Berl. and Vogl.

Macrophoma citrulli (B. and C.) Berl. and Vogl.; isolated by Dr. J. A. ELLIOTT from a cantaloupe (*Cucumis melo* L.) leaf procured from Alabama in the autumn of 1915.

Young pycnidia and small sclerotia form in abundance, soon rendering the culture dark brown or black. Usually a single cell in a hypha becomes slightly swollen. It then divides into 2 short cells (fig. 38). These cells in turn divide by cross, longitudinal, and diagonal walls (figs. 39, 40). The swelling continues until the body becomes a rounded or oblong mass much darker than the light brown mycelium (figs. 46, 47). The cells immediately adjacent to the dividing mass may divide into short cells and send out hyphal branches (figs. 42–44). The outer cells of the mass may also send out branching hyphae. The hyphae from all these sources form a network about the enlarging mass. Sometimes the hyphal branches fuse with the mass, and in other cases they appear to

have no part in its formation. The body thus developed is the primordium from which the pycnidium arises. It continues to enlarge, becomes pear-shaped, forms an ostiole, and develops spores. This mode of development is meristogenous, with a slight modification in that short hyphal branches either fuse with or form an envelope about the primordium.

Less frequently a second method appears, in which short branches from main mycelial strands direct themselves toward a point. There these branches interweave, swell, and divide to form a network (fig. 48). This network is at other times formed by the looping back or snarling of hyphal branches. The tangle enlarges, becomes more tightly woven, then the cells divide, swell, and anastomose to form a pseudoparenchymatous mass (fig. 49). This method is symphogenous. This species gives rise to both meristogenous and symphogenous developments.

The pycnidia of the species studied in the genera *Phoma* and *Macrophoma* indicate that both meristogenous and symphogenous methods of development may be found. These species present in the main the simple meristogenous mode in which a few cells of a single hypha take part (figs. 1, 6, 15, 23, 29, 38, 44); also a slight variation of the simple development in which hyphal branches both from the dividing cells and from other cells of the mother hypha form a network about the original cells taking part in the development (figs. 36, 39, 47). The compound meristogenous development is that in which 2 or more main hyphae, lying parallel or crossing, begin to divide into short cells at a point of contact, swelling and dividing to form a primordium (figs. 18, 22, 37). A few adjacent cells of each hypha and some surrounding branches take part in this type of development.

Sphaeronaema Fries

Sphaeronaema fimbriatum (E. and H.) Sacc.; procured from Dr. Byron D. Halsted, October 1916.

Halsted and Fairchild (18) report and figure the development of the pycnidium of *Ceratocystis fimbriata* as follows:

> In its initial stages the pycnidium arises as the swollen and curled or twisted tip of a vegetative hypha, or as a twist or knot in a sporophore between

the conidium and its point of union with the main hypha. Although observed to be present in numerous cases, no anastomosing of different hyphae branches seems necessary. Almost simultaneously with the first curving of the hypha tip side branches arise, which, by their growth and formation of septa, form the coarsely cellular membranous wall of the pycnidium.

Single mountings from plate cultures gave all stages of development verifying this description. The usual development was the twisting, budding, branching, and enlarging by cell division of single twisted hyphal branches (figs. 50–52). This is a variation of the meristogenous development.

SPHAEROPSIS Léveillé

Sphaeropsis malorum Pk.; isolated from apple fruits (*Pyrus malus* L.) from Centerville, Indiana, in October 1916.

HESLER (19) gives the development of the pycnidium of *Physalospora cydoniae* Arnaud in agar cultures as follows:

> The dense pseudoparenchyma of the maturer fruit bodies suggests meristematic divisions, but apparently the structure, for the most part, arises symphogenetically. In agar cultures a group of threads may be observed to be directed toward a common point where the pycnidium is to be formed. Here the hyphae are composed of cells 6–7 μ broad, their length varying from 20 to 70 μ, always longer than broad. In the region where the pycnidium is to be developed, the cells become noticeably shorter by the laying down of new walls; the cells also increase in diameter by growth, and the hyphae increase their numbers by branching. The interspaces found in the earlier stages are filled by the growing in of these branches and by a budding-like action of the hyphal cells bordering the space.

Among my own notes upon *Sphaeropsis malorum* Pk., made before the appearance of this description, is an account of the development of the primordium which it seems worth while to give here.

The pycnidial primordium of this species is formed usually according to the method designated by DEBARY as symphogenous. A number of hyphal branches interweave near their ends (fig. 53); these in turn branch and the branches also interweave (figs. 54, 55). The central portion becomes a tightly woven mass. The whole continues to enlarge until a spherical mass is formed, which develops into a pycnidium. Most of these primordia form deeply

imbedded in the agar, although a few develop on or near the surface. Those developing imbedded in the agar are smooth, while those on the surface have a fuzzy appearance. A very few pycnidia form by the compound meristogenous method described for *Phoma*. These usually form 2 or more contiguous hyphae in which a few adjacent cells divide, swell, continue to divide, and enlarge until a primordium similar to the one described is formed.

Sphaeropsis citricola McAlpine; isolated at Urbana, Illinois, January 1916, from a kumquat fruit (*Fortunella margarita* Swingle) from Lake City, Florida. The condition produced on the rind of the fruit was that of a very black carbonaceous spotting which finally spread over the whole fruit.

In culture a coarse brown mycelium 5–8 μ in diameter is produced. Numerous pycnidia 80–175 μ in diameter develop from primordia each of which arises either from a single cell or a few adjacent cells in a single hypha. This cell or group of cells (figs. 56, 57) divides by cross and diagonal walls (figs. 58–61), later by the addition of longitudinal walls to form a rounded or elongated mass (figs. 62, 63) which is slightly darker in color than the mycelium. This mass becomes globose, light brown, carbonaceous and reticulated, and is the primordium. Development is usually simple meristogenous. The compound meristogenous mode is seldom found (figs. 64–66). From pycnidia developing from these masses small hyaline spores 5–7×4–5 μ exude.

The genus *Sphaeropsis* presents a variation of the symphogenous development in that the branches interweave near their ends at a point some distance from the main mycelial strands (fig. 53). It also shows the more usual symphogenous type (figs. 54, 55) described for *Macrophoma* (figs. 48, 49). Simple meristogenous development occurs in one species. The compound mode seldom occurs in the species studied.

Coniothyrium Corda

Coniothyrium pyriana (Sacc.) Shel.; pure culture isolated from twigs of apple (*Pyrus malus* L.) from Savoy, Illinois, October 1916.

A few adjoining cells of a main hypha become slightly swollen and divide into short cells (figs. 67, 68). These cells swell, divide

by cross, diagonal, and longitudinal divisions, and continue to swell and send out short hyphal branches by budding (figs. 69, 70). This body increases in size, becoming darker in color. Branches from adjoining cells or closely lying hyphae may interweave with the branches from the mass, at length forming a primordium (fig. 71). from which a pycnidium develops. Usually this primordium arises from a single main hypha, but occasionally 2 or more contiguous hyphae are included in its formation. The mode of development is meristogenous, with small hyphal branches at times included in the formation. Both simple and compound modes are found, but the compound mode seldom occurs.

Coniothyrium, species indet.; culture procured from the air of the laboratory in the summer of 1917 by W. S. BEACH.

A few adjacent cells in a single hypha divide into shorter cells. These cells branch profusely (fig. 72). A few of the cells continue to divide and swell, and the branching hyphae divide and branch (fig. 73). The whole becomes a more closely formed mass (fig. 74) which forms a globose, dark brown, finely reticulated primordium. From this the pycnidium develops. The mode of development is simple meristogenous.

The genus *Coniothyrium*, so far as studied, presents only the meristogenous development.

SEPTORIA Fries

Septoria polygonorum Desm.; isolated from its host *Polygonum persicaria* L. at Urbana, Illinois, July 1916.

The development of the primordium in most cases is by the usual meristogenous method, but in some instances it is atypical. Some of the primordia arise from a single hypha, 1 or 2 cells of which divide transversely and diagonally, and, swelling slightly, continue to divide (fig. 75). Later this mass develops into a globose, finely reticulated, pale brown body. In some instances the compound mode appears in which 2 or more hyphae are involved in the formation (fig. 76). In other cases primordia are to be found arising from a main hyphal strand with numerous branches of nearby hyphae intermingling in the mass (fig. 77). This involves both the meristogenous and the symphogenous

methods, and so may be considered a combination of the two. The principal method of development is meristogenous.

Septoria scrophulariae Pk.; isolated from its host *Scrophularia leperella* Bicknell by Mr. WALTER S. BEACH during the summer of 1917.

In pure culture it produces a closed sporing body, the primordium of which arises either from a few cells of a single hypha or from 2 or 3 closely lying hyphae (figs. 78, 79). These primordial cells divide, swell, and continue to divide to form a small ball-like structure which later develops into 1 or more pycnidia. They thus arise either by the simple or compound meristogenous mode.

Septoria helianthi E. and K.; isolated from its host *Helianthus grosseserratus* Mortens by Mr. WALTER S. BEACH in the summer of 1917 at Urbana, Illinois.

Pycnidia form readily in culture. A single cell or a few adjoining cells in a hypha become slightly swollen. These then divide into shorter cells which swell and send out very short branches (fig. 80). The swelling continues until a rounded mass is formed, slightly darker in color than the pale brown mycelium (fig. 81). The body thus formed becomes almost globose in shape. The outer portion or covering becomes membranous with a cellular appearance. This primordium continues to enlarge, and becomes ovate or elliptical in shape. The development is simple meristogenous, within a single strand of mycelium.

The genus *Septoria* can hardly be judged by these few species, but these, with those reported in the literature, indicate that the 2 main methods of development, namely, meristogenous and symphogenous, and even a combination of these two, may occur.

SPHAERONAEMELLA Karst.

Sphaeronaemella fragariae Stevens and Peterson; procured from Dr. ALVAH PETERSON, Urbana, Illinois.

A few cells of a single hypha divide, producing very short cells. These bud and branch profusely, usually in one direction, into short hyphae (fig. 82). These branches anastomose (fig. 83), twining about in a circular manner. They then divide and swell, forming a small mass which usually protrudes from one side of the main

mycelial strand from which it arises (fig. 22). The body thus formed is the primordium from which the pycnidium arises.

In the genus *Sphaeronaemella* a striking variation of the meristogenous development is found. A few cells of a main mycelial thread begin to divide as in the simple meristogenous mode, but short hyphal branches arise from these, usually on one side, curling and twisting about each other to form a ball-like mass. In some cases this mass envelops and includes the main hypha (fig. 84).

GLOEOSPORIUM Desmazières and Montaigne

Gloeosporium rufomaculans (Berk.) Thüm.; isolated from fruit of apple (*Pyrus malus* L.) from Neoga, Illinois, September 1917.

The primordium of the acervulus arises from a number of neighboring hyphae. These branch into short hyphae which branch in turn, forming a loosely woven network. A spreading tuft arises from this loosely woven base (fig. 87). This cushion with its tuft of short hyphae is the primordium. It usually originates symphogenously, sometimes meristogenously.

Gloeosporium musarum C. and M.; isolated from a banana (*Musa sapientum* L.) from the Champaign market, July 28, 1916.

Short hyphal branches from main mycelial strands interweave near their ends. Other hyphae intertwine about this initial portion. Some branches fold or loop back upon themselves, while still others branch again. The cells of the interwoven mass divide, swell, and branch (fig. 88). At length a cushion-like base is formed. This is the primordium of the acervulus, from which short conidiophores arise which bear conidia. In terms of pycnidial development it arises symphogenously. In culture this fungus bears many spores, either sessile or upon short conidiophores outside of acervuli, scattered freely upon the mycelium. These spores appear before the formation of acervuli, making the study of the beginning of acervuli difficult.

COLLETOTRICHUM Corda

Colletotrichum lagenarium (Pers.) E. and H.; isolated from a watermelon (*Citrullus vulgaris* Schrad.) procured on the Champaign market, September 1917.

The primordia of the acervuli begin their formation deeply imbedded in the media, even near the bottom, by the time the culture is 4 cm. in diameter and no more than 4 or 5 days old. At this age no conidia have developed upon the short conidiophores or as buds from cells of hyphae, as often occurs when the culture becomes older. The primordia arise by 2 methods: (1) from a few cells of a main hypha arise a few or many short budlike hyphae (figs. 89–92); these elongate, intertwine, and branch to form a cushion-like base from which very short conidiophores arise; (2) hyphal branches from a few neighboring or contiguous mycelial strands intertwine, some of the threads forming short loops which mass, intertwine, and branch (figs. 93–96), forming an irregularly shaped, loosely made, cushion-like base from which conidiophores arise as in the other type. Irregular large acervuli, or acervuli-like groups, bearing numerous conidia, arise in this latter type. The first mentioned type may be considered as meristogenous, both simple and compound modes appearing; the second is symphogenous.

The genera *Gloeosporium* and *Colletotrichum* have been extensively studied by SHEAR (**36**), STONEMAN (**42**), SOUTHWORTH (**38, 39**), EDGERTON (**11**), and others. Late stages of development and cross-sections of different stages have been figured from fixed material in host tissues, but little has been said in regard to the early development of the acervulus. The 3 species studied give insight only into the origin of the cushion-like base from which the acervulus arises. They present 2 distinct types, the simple loosely woven base that arises from a single hypha or from a few contiguous hyphae, and the more complexly interwoven base which arises symphogenously.

PESTALOZZIA De Notaris

Pestalozzia palmarum Cke.; pure culture procured from the Centralstelle für Pilzkulturen, Amsterdam, Holland.

A few hyphae, usually 2–6, lying side by side form a bed in which a few cells begin to divide into shorter cells. These cells may be in only one of the hyphae (fig. 97*a*), or they may be contiguous cells of 2 or more hyphae (fig. 100). These cells swell and continue to divide by cross and longitudinal walls, at length forming

a protruding oval or obovate body (fig. 97*b*) which is the primordium of the pycnidium-like body which later forms. This primordium arises by either simple (fig. 97*a*) or compound meristogenous (fig. 100) development. It soon becomes globose and membranous, with a cellular outer wall, but remains light of color, so that a few dark spores may be seen within (figs. 98, 99). At this stage the structure is to all appearances a young pycnidium (fig. 101). Within a few days the top breaks, the few spores already formed are extruded, sometimes rather forcibly (fig. 102), and the cavity then becomes saucer-shaped. Conidiophores arise in profusion, and many spores are produced. When the first spores are noted the body is a pycnidium, if observed in a later stage it appears like an acervulus. In *Pestalozzia capiomanti* similar facts were noted by BAINIER and SARTORY (**4**), while LEININGER (**24**) in his studies of *P. palmarum* speaks of these bodies as pseudopycnidia.

Pestalozzia guepini Desm.; isolated from the fruit of a kumquat (*Fortunella margarita* Swingle) from Lake City, Florida, January 1916.

A few pycnidia were produced in plate culture. In the beginning stages a number of branches of closely lying or nearby hyphae branch, snarl, and intertwine (figs. 103, 104). Cells within the more closely twined part of the mass swell and divide by continued cross and longitudinal divisions until a rounded mass is formed, which is the primordium of the pycnidium (fig. 105). This primordium arises symphogenously. The development of the final sporing body from this stage is very similar to that described for *Pestalozzia palmarum*.

Pestalozzia, species indet.; isolated, by the author November 1916, from leaves of peony (*Paeonia officinalis* Retz) procured by Dr. H. A. ANDERSON near Crawfordsville, Indiana.

A good growth of mycelium and numerous sporing bodies were produced in plate cultures. The mycelial threads are slightly larger than those of other species of *Pestalozzia* studied. Pycnidial primordia arise usually by the compound meristogenous method (figs. 107, 108). As these bodies develop from the pycnidial stage to the open type, the formation of spores within (fig. 109), a

breaking open of the pycnidium, and its further development into what appears to be an acervulus may be seen.

Pestalozzia, species indet.; pure culture procured from Dr. G. P. CLINTON, New Haven, Connecticut, in November 1916. It had been isolated from dead maple (*Acer*) bark in October 1910.

This *Pestalozzia* was the most vigorous of all those cultured. Sporing bodies were produced in abundance. Two or 3 contiguous hyphae, or in some cases as many as 10 or 12, take part in the formation of the primordium. A few cells continue to swell and divide until a small mass is formed (fig. 110). They branch slightly (fig. 111) until a primordium of tissue-like type is formed. It is of compound meristogenous development, especially pronounced in cases where 10 or more hyphae take part. The young pycnidia-like bodies continue to develop, produce spores, then break open (fig. 112) and change into the acervulus form as in the previous species mentioned.

In *Pestalozzia* a condition is found that is quite distinct from any other acervulus-forming fungus studied, in that it first produces a sporing body which morphologically is a pycnidium. These pycnidia arise by any of the various modes previously described. The different species vary in the manner of development, but whatever the method there first appears a pycnidium which later breaks open and becomes acervulus-like.

PATELLINA Speg.

Patellina fragariae Stevens and Peterson; pure cultures procured of Dr. A. PETERSON, September 1916. It was also isolated from strawberries (*Fragaria chiloensis* Duschesne) from Centerville, Indiana, June 1916.

This fungus forms numerous sporodochia in concentric rings in plate cultures. These sporodochia arise in 2 rather typical ways. A few cells of a hypha divide into very short cells. These cells swell and bud, producing numerous branches in some cases (fig. 115), in other cases a tuft of 2 or 3 branches. These branches elongate slightly to form a pedicel (fig. 116), then branch at the tips and radiate to form a distinct urnlike body (fig. 117), within which a cushion or bed forms and gives rise to the conidiophores. The

sporodochia are usually produced singly by a simple meristogenous mode. In other instances sporogenous areas develop, and in these 1 or 2 cells, in each of the closely lying hyphae, branch profusely (fig. 113). Many of these branches unite into a mass and, without the formation of a definite pedicel and peridium (fig. 114), give rise to hundreds of conidiophores.

Patellina, species indet.; from a quince (*Cydonia vulgaris* L.) procured on the Champaign market, November 1917.

Strands of 6–20 hyphae are formed, and at some definite point within the strand a few adjacent cells branch; thus a tuft of a number of branches is formed (fig. 118). This tuft becomes slightly larger at the upper end by continued branching, while the lower portion constitutes a pedicel consisting of a few large branches, where a few hyphae enter into its formation. A more substantial closely formed pedicel or base is present if a larger number of hyphal branches are concerned in its origin (figs. 118, 119). The upper half or less becomes cuplike, and the outer hyphae curve inward as a superficial covering, while within conidiophores and conidia are formed. The type is compound meristogenous.

In *Patellina* the sporodochium develops in a very characteristic manner, arising by the meristogenous method. A single isolated mycelial thread gives rise to a very simple sporodochium. If a number of mycelial strands are crowded together, each branches characteristically, and the branches form a sporodochium of the compound type. The type of sporodochium varies with the type of base formed.

Volutella Tode

Volutella fructi S. and H.; procured from Mr. Wilmer G. Stover, Ohio State University. It was isolated by G. C. Meckstroth in the winter of 1916 from a fruit of apple (*Pyrus malus* L.) grown in western Ohio.

The sporodochia form in abundance in plate cultures, the primordium arising from a main hypha. A definite portion, 1, 2, or 3 cells in length, takes on a brownish color. These cells divide and some of them branch, usually from one side only (figs. 120,

124), forming a small tuft (figs. 120, 125), the branches of which elongate and then divide again, forming a larger tuft (figs. 121, 122). The first interwoven branches form a short pedicel. This cuplike structure may be regarded as the primordium of the sporodochium (fig. 123), from which a bed of short conidiophores is formed. The development is clearly simple meristogenous. Other larger bases are quite often formed symphogenously. Then hyphal branches from all adjacent mycelial threads interweave and anastomose, forming a black mass of sclerotial character from which hyphae arise and interweave into a cuplike bed from which conidiophores develop.

Volutella circinans (Berk.) Stevens and True; from a white globe onion (*Allium cepa* L.) at Urbana, Illinois, September 1917.

Primordia arise by either of 2 methods, simple meristogenous (fig. 126) or symphogenous (figs. 128–130). In most instances a black stroma-like mass of interwoven hyphae is formed symphogenously (figs. 130, 131), from which the sporodochium later develops. Setae may be found arising as hyphal branches (fig. 127).

Volutella is very similar to *Colletotrichum* and *Gloeosporium* in the origin of the sporing bodies; the meristogenous and symphogenous methods are both found. The principal distinction is in the formation of a more compact and usually larger base or subicle upon which the sporodochium is produced. The origin of this subicle if it is simple is usually meristogenous, but if a complex subicle is formed it arises symphogenously by the interweaving of numerous hyphal branches.

Epicoccum Link

Epicoccum, species indet.; isolated from a plate culture in which it appeared as a contamination in October 1917.

The sporing body of this fungus arises from 2 or more closely lying hyphae which produce erect branches from a rather localized area. Two or more such hyphae arise which branch in turn near their tips. These form a spreading tuft of conidiophores each bearing a conidium at its end. This is a simple form of sporodochium (fig. 132). The development is compound meristogenous.

PYCNIDIAL STAGE OF MELIOLA(?) CAMELLIAE (Catt.) Sacc.

This pycnidium was studied from herbarium material collected by Dr. F. L. STEVENS in Porto Rico. The fungus, which usually is reported as *M. camelliae* and is perhaps better known as "sooty mold" (**48**), is plainly not a true *Meliola*, as the genus is limited by recent writers (**16, 40**), and the position of its ascigerous stage has not been definitely determined.

A small pycnidium arises from a single cell of the main hypha or a single cell of a hyphal branch. The initial cell is usually an intermediate cell, but it may be a terminal one. In either case it divides by transverse and diagonal walls into 2, then 4 cells (fig. 133), and by swelling and dividing becomes an elliptical, finely reticulated, dark brown body (figs. 134–137). The mode is simple meristogenous.

These observations do not essentially disagree with the descriptions as given by ZOPF (**53**) and TULASNE (**43**), who studied early stages of the development of the pycnidium of the sooty molds.

Discussion

Two main methods of origin and early development are found in pycnidial formation, namely meristogenous and symphogenous. The meristogenous method resolves itself into 2 modes, simple (figs. 1, 2, 4, 5) and compound (figs. 18, 22, 37). In the simple mode the pycnidium develops from a single cell or a few adjacent cells of a single hypha. In the compound mode adjacent cells of 2 or more contiguous hyphae divide, swell, and sometimes branch, all of these then anastomosing freely to form a pseudoparenchymatous mass. Variations of these 2 modes are found in *Macrophoma citrulli* (figs. 39, 43, 47), *Coniothyrium pyriana* (figs. 69–71), *Septoria polygonorum* (figs. 76, 77), *Sphaeronaema fimbriatum* (figs. 50–52), and *Sphaeronaemella fragariae* (figs. 82–85).

The symphogenous method (figs. 48, 49, 54, 55) is less often found in the species studied. In this method of development, branching hyphae from main mycelial threads are directed toward a common point, loop back, and interweave to form a loose network which later becomes more close. The hyphae of this ball anastomose into a pseudoparenchymatous mass from which the pycnidium develops.

Table I gives the species and the methods of development found in each of the pycnidium-forming species. *Phoma herbarum*, *Phoma pirina*, *Phoma* from clover, *Phoma* from grape, *Sphaeronaema fimbriatum*, *Coniothyrium* species indet., *Septoria helianthi*, *Sphaeronaemella fragariae*, and a pycnidium of *Meliola*(?) *camelliae* show a simple meristogenous origin and development, and other methods of development seldom or never appear.

TABLE I

Species	Simple meristogenous	Compound meristogenous	Symphogenous
Phoma herbarum West	+	−	−
Phoma destructiva Plowr	+	+	−
Phoma pirina (Fries) Cooke	+	−	−
Phoma from clover	+	−	−
Phoma from grape	+	−	−
Phoma cichorii Passr	+	+	−
Macrophoma citrulli (B. and C.) Berl. and Vogl	+	+	+
Sphaeronaema fimbriatum (E. and H.) Sacc	+	−	−
Sphaeropsis malorum Pk	−	+	+
Sphaeropsis citricola McAlpine	+	+	−
Coniothyrium pyriana (Sacc.) Shel	+	+	−
Coniothyrium, species indet	+	−	−
Septoria polygonorum Desm	+	+	+
Septoria scrophulariae Pk	+	+	−
Septoria helianthi E. and K	+	−	−
Sphaeronaemella fragariae S. and P	+	−	−
Pycnidium of Meliola (?) camelliae (Catt.) Sacc	+	−	−

In *Phoma destructiva*, *Phoma cichorii*, *Sphaeropsis citricola*, *Coniothyrium pyriana*, and *Septoria scrophulariae* the pycnidial primordia arise by either the simple or compound meristogenous modes, the simple mode being the more common.

Macrophoma citrulli, *Sphaeropsis malorum*, and *Septoria polygonorum* give rise to their pycnidial primordia by either the symphogenous or meristogenous methods. In *Sphaeropsis malorum* the symphogenous method is the main one. The compound meristogenous method appears occasionally. In *Macrophoma citrulli* the simple meristogenous mode prevails, but the others are often found. In *Septoria polygonorum* the compound meristogenous mode is more often found, although a few primordia arise by the symphogenous method, and occasionally the simple meristogenous mode appears.

Variations of these 3 modes of development are found. No 2 species give exactly the same development. In symphogenous development a few branches from nearly hyphal strands may interweave near their ends, or intermingling hyphae may loop back, branch, and interweave or snarl into a knotlike mass. In the simple meristogenous mode the origin may be a single cell which swells and divides, or a number of cells that swell and divide simultaneously. In some cases branches arising from the dividing cells anastomose with the enlarging mass. In other cases branches arise from more distant cells of the same strand and take part in the development. In a few instances short budlike branches arise from a few cells of a hyphal strand, enlarge, divide into short cells, intertwine, and anastomose to form a pycnidium.

Neither have the acervuli-forming fungi in the Melanconiales been studied as to the early development of their sporing bodies, nor has the origin of the sporodochium been given special attention. SHERBAKOFF (**37**), in studies of *Fusarium*, describes a simple type of sporodochium found in cultures. TULASNE (**43**), STONEMAN (**42**), SOUTHWORTH (**38, 39**), WOLF (**50, 51**), and others figure and describe later stages of the development of acervuli, and the later development of sporodochia are referred to in the literature, but the subject is considered merely incidentally.

The fungi that form acervuli and sporodochia may be classed according to the manner of origin and development of the primordia on the same basis as the pycnidia-forming species.

In table II fungi of the Melanconiales and Tuberculariaceae that were studied are listed, indicating the method or methods of development of the primordia. In *Gloeosporium rufomaculans*, *Gloeosporium musarum*, and *Pestalozzia guepini* the primordia originate by the symphogenous method. In *Colletotrichum lagenarium* and *Volutella circinans* the symphogenous method prevails, although the simple meristogenous mode occurs occasionally. The compound meristogenous mode is well exemplified by *Pestalozzia palmarum*, *Pestalozzia* from peony and from maple. *Epicoccum* and *Pestalozzia* from maple have only the compound meristogenous mode. The simple meristogenous method of development appears in *Colletotrichum lagenarium*, *Pestalozzia palmarum*, *Pestalozzia*

from peony, *Volutella fructi*, and *Volutella circinans;* it also is at times observed in the development of isolated sporodochia of *Patellina fragariae*. This mode is more seldom found than either the compound meristogenous or symphogenous method in the development of acervuli and sporodochia.

TABLE II

Species	Simple meristogenous	Compound meristogenous	Symphogenous
Gloeosporium rufomaculans (Berk.) Thüm.	−	−	+
Gloeosporium musarum C. and M.	−	−	+
Colletotrichum lagenarium (Pers.) E. and H.	+	+	+
Pestalozzia palmarum Cke.	+	+	−
Pestalozzia guepini Desm.	−	−	+
Pestalozzia from peony	+	+	−
Pestalozzia from maple	−	+	−
Patellina fragariae S. and P.	+	+	−
Patellina from quince	−	+	−
Volutella fructi S. and H.	+	−	+
Volutella circinans (Berk.) S. and T.	+	−	+
Epicoccum, species indet.	−	+	−

The species of *Pestalozzia* studied present a type of sporing structure which is in need of further investigation in other genera and species. From the mature structure it has been classed as an acervulus, but from its origin and development it is not a true acervulus, for it arises as a pycnidium and opens to form an acervulus when mature. This may be called a pseudo-acervulus.

According to POTEBNIA (30) and DIEDICKE (10), some species of *Septoria* and *Ascochyta* have open-topped sporing bodies arising by the interweaving of hyphae to form a bed from which arises a peridium partially surrounding the inner sporing surface. Such a structure is designated by POTEBNIA as a pseudo-pycnidium.

Study of the origin and development of the sporing bodies of the many genera and species of the Sphaeropsidales and Melanconiales which have not yet been investigated will no doubt add to these 2 types.

In the pycnidia-bearing fungi studied the meristogenous method of development is the more prevalent, the symphogenous type seldom appearing. In species forming acervuli and sporodochia,

the tendency is toward the more complex methods, especially if *Pestalozzia* be regarded as belonging to the Sphaeropsidales. As was to be expected, in the pycnidial development no sexual organs, ascogenous hyphae, or nuclear fusions were observed.

Summary

1. Pycnidia originate and develop by 2 main methods, namely, meristogenous and symphogenous.

2. The meristogenous method resolves itself into 2 modes, simple and compound.

3. Variations of the meristogenous method are found, for example, in *Coniothyrium pyriana* and *Sphaeronaemella fragariae*.

4. The symphogenous method is less often found and is variable.

5. Acervuli arise in the same manner as do pycnidia, simple acervuli by the simple meristogenous mode, and complex ones usually by the compound meristogenous or symphogenous method.

6. Complex subicles usually arise symphogenously, although they may arise by the compound meristogenous mode.

7. Simple sporodochia, especially those appearing on single isolated strands, originate by the simple meristogenous method.

8. Complex sporodochia, with a large base or subicle, usually arise either by the compound meristogenous mode or symphogenously.

9. The pseudo-acervulus of the species of *Pestalozzia* studied arises and develops as a pycnidium which breaks open and appears like an acervulus.

10. The simple meristogenous development is the more often found in the Sphaeropsidales, while the compound meristogenous and symphogenous modes are the more usual in the Melanconiales and Tuberculariaceae.

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EXPLANATION OF PLATES XVII–XXII

The drawings were made with a Leitz camera lucida and a Leitz one-twelfth oil immersion lens or a no. 6 objective. The scale appears on the plates.

PLATE XVII

Phoma

FIG. 1.—*P. herbarum* West: 4-celled stage of pycnidial primordium of simple meristogenous origin.

FIG. 2.—Slightly later stage.

FIG. 3.—Stage similar to fig. 2.

FIG. 4.—Young pycnidium with light colored rostrum where ostiole will form.

FIG. 5.—Mature pycnidium and spores formed by simple meristogenous development.

FIG. 6.—*P. pirina* (Fries) Cooke: few-celled stage; simple meristogenous origin.

FIG. 7.—Slightly later stage.

FIG. 8.—Many-celled pseudoparenchymatous stage with branches protruding from mass.

FIG. 9.—Irregular mass from which pycnidium develops.

FIGS. 10, 11.—Few-celled stages in origin of which 2 or more hyphae are involved; compound meristogenous origin.

FIG. 12.—Similar to fig. 11, but slightly older.

FIG. 13.—Primordium in which a 3-parted hyphal strand is involved.

FIGS. 14–16.—*P. destructiva* Plowr.: beginning stages in pycnidial development; simple meristogenous origin.

FIG. 17.—Slightly later stage.

FIGS. 18, 19.—Compound meristogenous development in which 2 or more hyphae are involved.

FIG. 20.—Development near end of hypha with branches budding from mass.

FIG. 21.—Irregular meristogenous development.

FIG. 22.—Typical compound meristogenous development with 3 parallel hyphae involved.

PLATE XVIII

FIGS. 23, 24.—*Phoma* from clover: beginnings of pycnidia.

FIG. 25.—Slightly older stage with a few short budding branches.

FIGS. 26, 27.—Later stages with one end enlarging slightly.

FIG. 28.—*Phoma* from grape: mycelial threads with different stages of pycnidial development.

FIG. 29.—Beginning stage: simple meristogenous.

FIGS. 30–32.—Later stages.

FIG. 33.—Mature pycnidium.

FIG. 34.—*P. cichorii* Passr.: early stage in development; short cells formed which divide and branch.

FIG. 35.—Early stage in which is much branching.

FIG. 36.—Medium stage in simple meristogenous development.

FIG. 37.—Typical compound meristogenous development.

Macrophoma

FIG. 38.—*M. citrulli* (B. and C.) Berl. and Vogl.: 2-celled stage in origin of pycnidium, from drop culture; simple meristogenous development.

FIG. 39.—Same 12 hours later.

FIGS. 40, 41.—Early stages of other pycnidia, from drop culture; slight variation from simple meristogenous type in that many branches are involved.

FIGS. 42, 43.—Early stages in which much branching takes place; drop culture.

FIGS. 44, 45.—Early stages from Petri dish culture.

FIGS. 46, 47.—Later stages.

PLATE XIX

FIG. 48.—*M. citrulli* (B. and C.) Berl. and Vogl.: symphogenous development in which branches from a number of main strands interweave.

FIG. 49.—Later stage in which a winding of the hyphae and cell division have taken place forming a pseudoparenchymatous mass.

Sphaeronaema

FIGS. 50–52.—*S. fimbriatum* (E. and H.) Sacc.: early stages of pycnidium in which a hypha coils, branches, and divides to form knotlike mass.

Sphaeropsis

FIG. 53.—*S. malorum* Pk.: Early stage in symphogenous development in which branches *a*, *b*, *c* interweave near their ends to form a ball.

FIG. 54.—Interwoven hyphae in early stage of symphogenous development.

FIG. 55.—Slightly later stage.

FIGS. 56, 57.—*S. citricola* McAlp.: very early stages in origin of simple meristogenous development of pycnidia.

FIGS. 58, 59.—Slightly later stages.

FIGS. 60–63.—Later stages with short hyphae branching from masses.

FIGS. 64–66.—Unusual examples of developments in which more than one hypha is involved; compound meristogenous.

Coniothyrium

FIGS. 67, 68.—*C. pyriana* (Sacc.) Shel.: early stages in development.

FIGS. 69, 70.—Later stages in which numerous branches from dividing mass are involved.

FIG. 71.—Pseudoparenchymatous mass from which a pycnidium arises.

PLATE XX

FIG. 72.—*Coniothyrium* species from laboratory air: early stage showing short cells and short branches as origin.

FIG. 73.—Slightly later stage in which cells and branches from main hypha divide into short cells and anastomose.

FIG. 74.—Stage slightly more developed than fig. 73; slight variation from simple meristogenous type.

Septoria

FIG. 75.—*S. polygonorum* Desm.: beginning stage of simple meristogenous development.

FIG. 76.—Development in which original hypha and numerous branches are involved.

FIG. 77.—Pseudoparenchymatous primordial mass formed by symphogenous method.

FIGS. 78, 79.—*S. scrophulariae* Pk.: early stages in compound meristogenous development.

FIG. 80.—*S. helianthi* E. and K.: early stage in beginning of simple meristogenous development.

FIG. 81. Later stage.

Sphaeronaemella

FIG. 82.—*S. fragariae* S. and P.: early stage of simple meristogenous development.

FIG. 83.—Later stage in which branches and original hypha have anastomosed to form a mass on one side of main strand.

FIG. 84.—Later stage in which mass surrounds main strand.

FIG. 85.—Late stage which has developed by a winding and dividing of hyphal branches from a few cells upon one side of main strand.

Gloeosporium

FIG. 86.—*G. rufomaculans* (Berk.) Thüm.: early stage of meristogenous development; this method rarely occurs.

FIG. 87.—Side view of an acervulus formed by symphogenous method: compressed so that cushion-like bed of conidiophores is pulled away from basal hypha.

FIG. 88.—*G. musarum* C. and M.: top view of partially developed acervulus; development symphogenous.

Colletotrichum

FIG. 89.—*C. lagenarium* (Pers.) E. and H.: very early stage in which acervulus originates in a few short branches from a single hypha.

FIG. 90.—Numerous budding branches from a few cells of one hypha forming beginning of an acervulus; simple meristogenous.

FIG. 91.—Early stage in development of acervulus.

FIG. 92.—Primordium in which a few branches are involved.

FIG. 93.—Early stage in symphogenous development: hyphal branches loop and interweave in this mode.

FIG. 94.—Same as fig. 93, 24 hours later.

FIG. 95.—Beginning stage of symphogenous development.

FIG. 96.—Later stage.

PLATE XXI

Pestalozzia

FIG. 97.—*P. palmarum* Cke.: *a*, very early stage in meristogenous development, a few cells divided and swelling; *b*, later stage.

FIGS. 98, 99.—Pycnidia with young spores developed within.

FIG. 100.—Primordium of compound meristogenous origin.

FIG. 101.—Young pycnidium at stage just before opening.

FIG. 102.—Pycnidium opening and extruding spores: following this stage cuplike interior formed, longer conidiophores develop, and body becomes a pseudo-acervulus.

FIGS. 103, 104.—*P. guepini* Desm.: early stages in symphogenous development.

FIG. 105.—Later stage; pycnidia of this species form spores within and later break open as pseudo-acervuli.

FIG. 106.—*Pestalozzia* from peony: meristogenously developed pycnidial mass.

FIG. 107.—Early stage in symphogenous development.

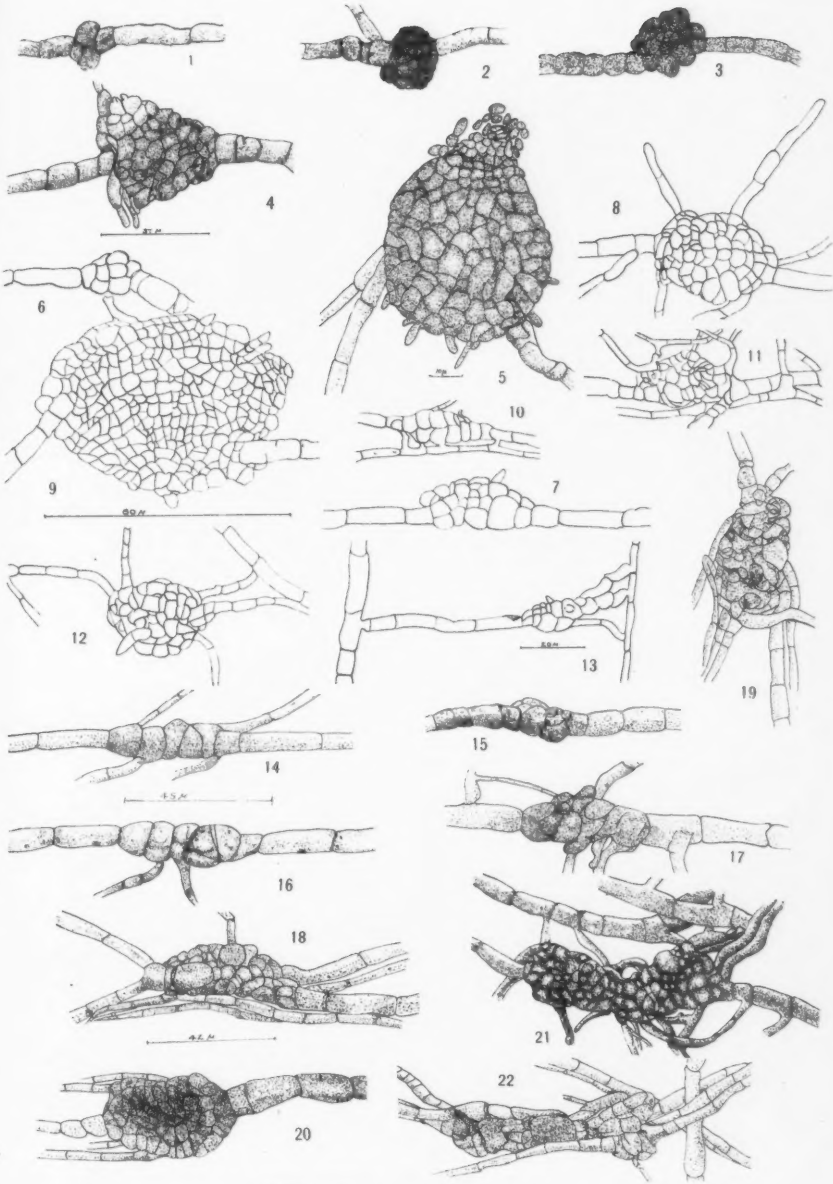
FIG. 108.—Later stage.

FIG. 109.—Pycnidium which has opened, surface view.

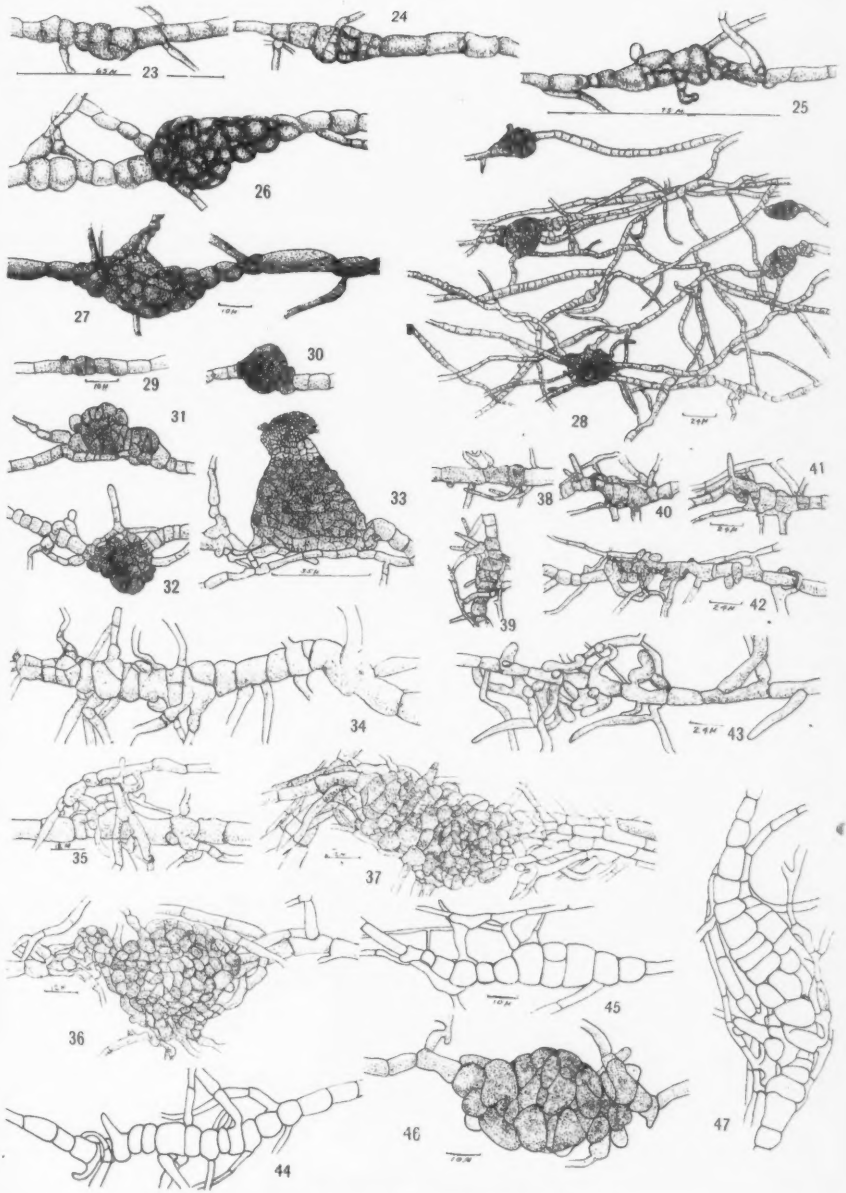
FIG. 110.—*Pestalozzia* from maple: compound meristogenous development from a number of parallel hyphae.

FIG. 111.—Later stage.

FIG. 112.—Young pycnidium breaking open on one side: spores show within.



KEMPTON on PYCNIDIUM



KEMPTON on PYCNIDIUM

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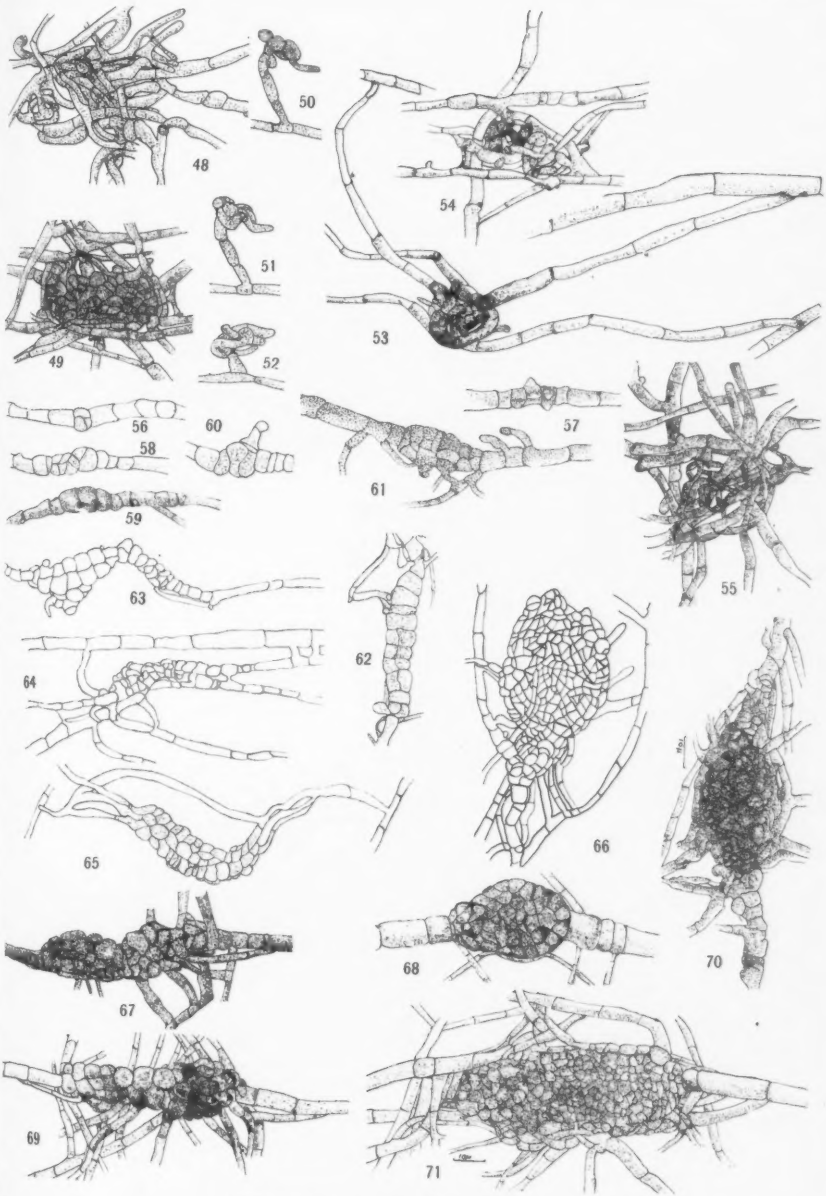

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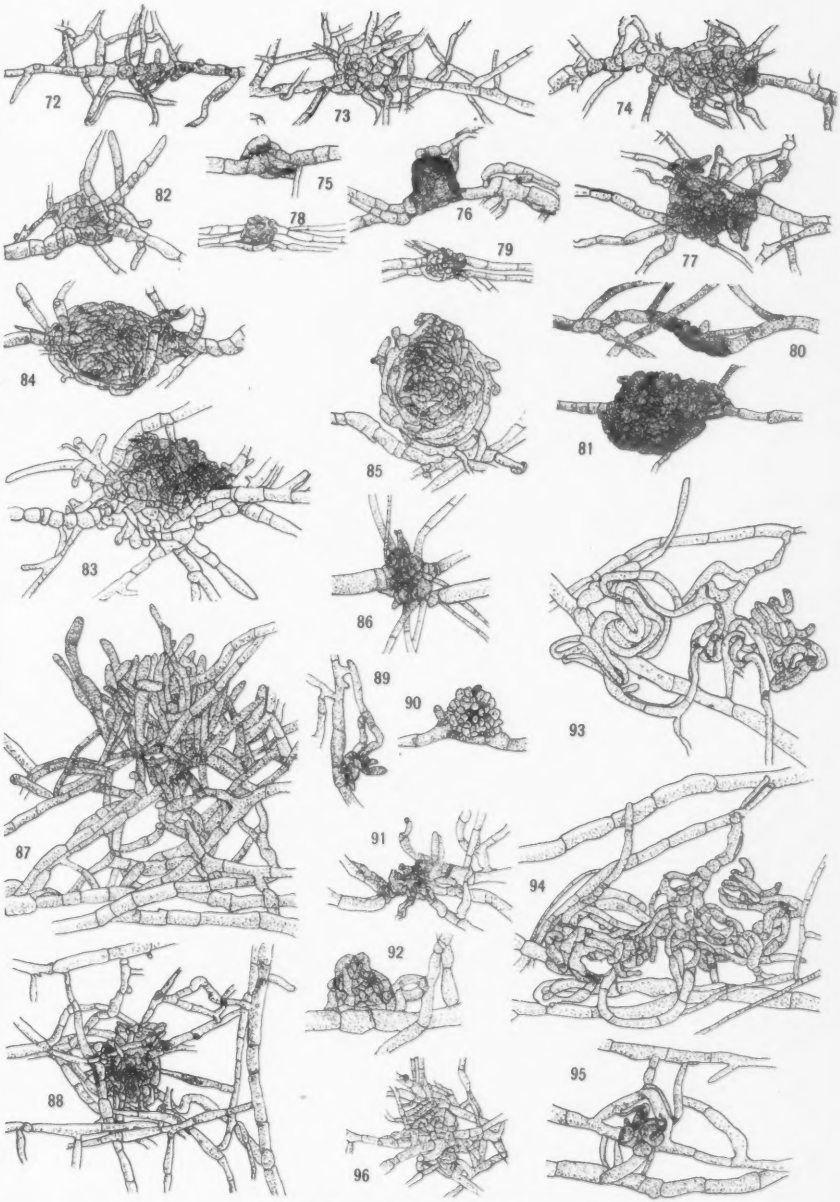

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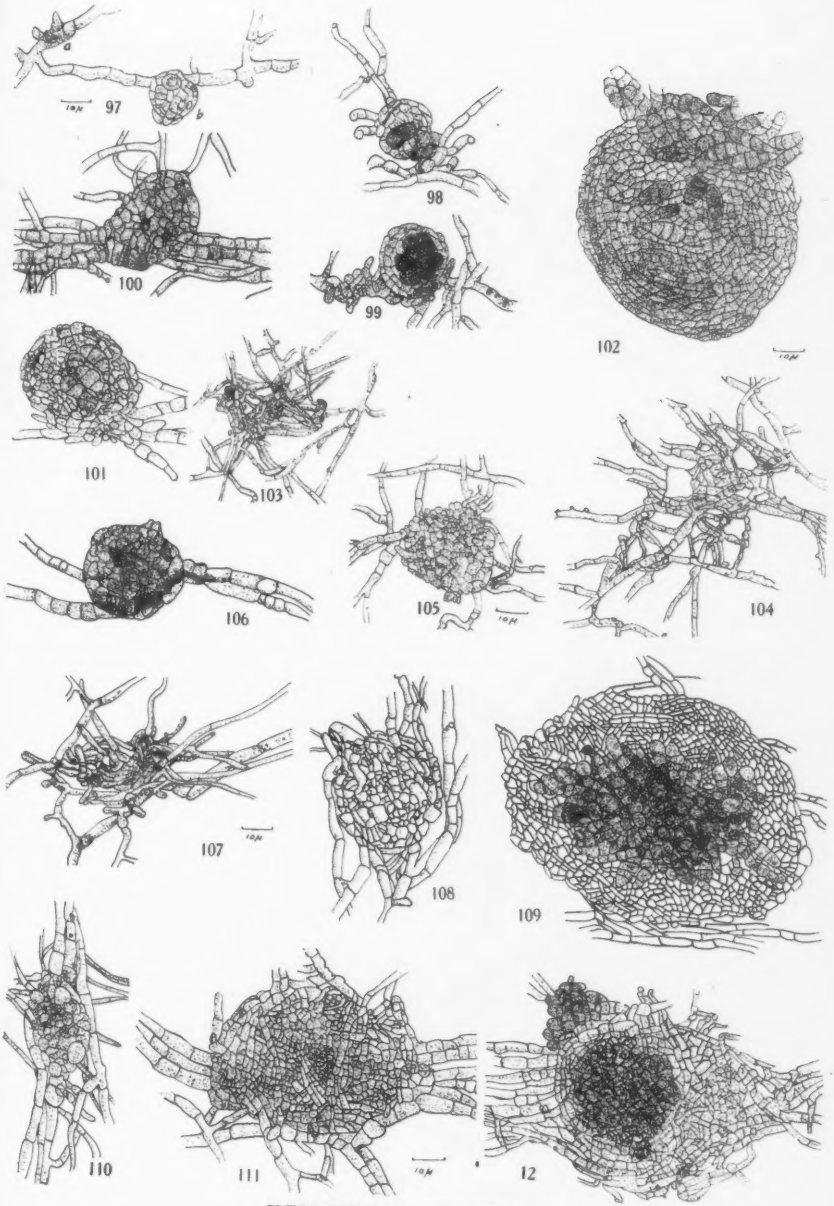

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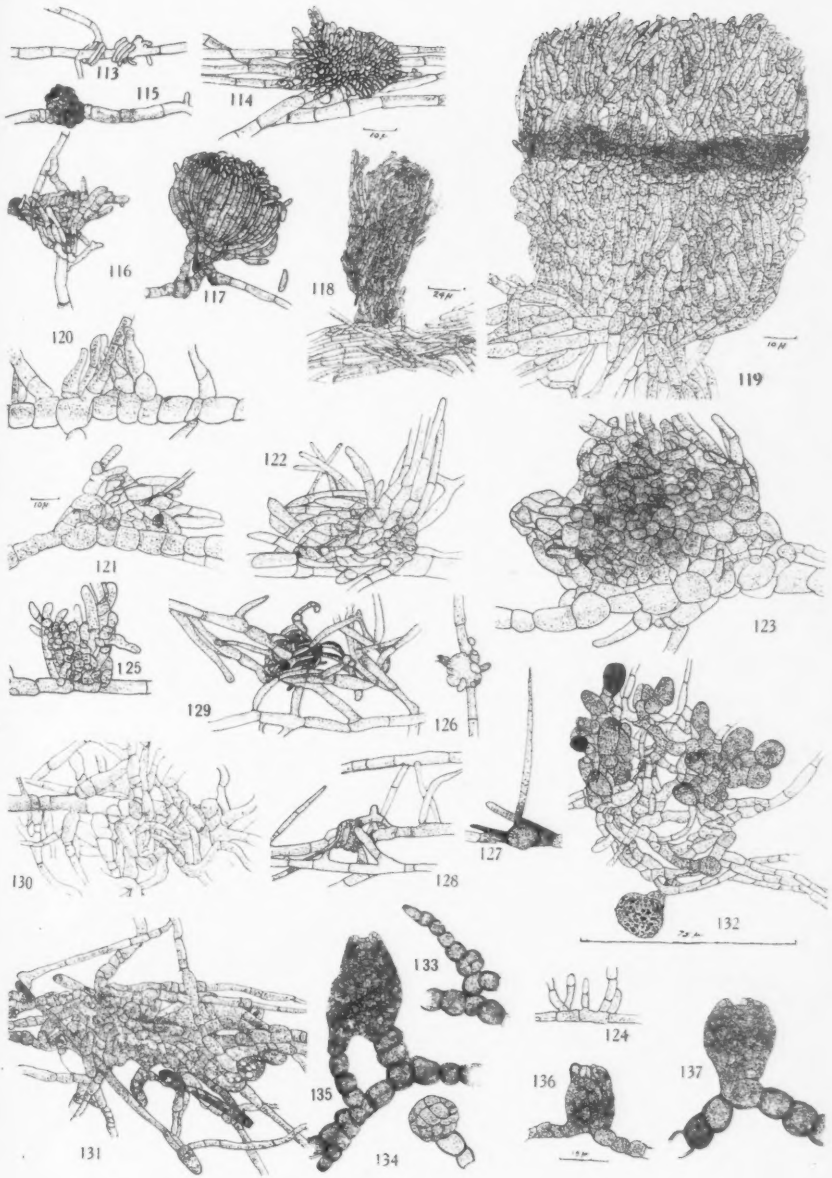

KEMPTON on PYCNIDIUM

PLATE XXII

Patellinia

FIG. 113.—*P. fragariae* S. and P.: single strand, with characteristic branching, from sporogenous area.

FIG. 114.—Compound meristogenously developed sporodochium produced from a number of hyphae, as fig. 113.

FIG. 115.—Simple meristogenous development with branches arising from a few cells.

FIG. 116.—Later stage in simple meristogenous development.

FIG. 117.—Simple, meristogenously developed, mature sporodochium.

FIG. 118.—*Pestalozzia* from quince: sporodochium of compound meristogenous development; spore bearing area develops from cuplike top.

FIG. 119.—Another view of sporodochium similar to fig. 118.

Volutella

FIG. 120.—*V. fructi* S. and H.: very early stage in simple meristogenous origin of a sporodochium; few cells branch to form base.

FIG. 121.—Slightly later stage.

FIG. 122.—Medium stage.

FIG. 123.—Fully developed base or subicle.

FIG. 124.—Same as fig. 120: smaller hypha.

FIG. 125.—Later stage than fig. 124.

FIG. 126.—*V. circinans* Stevens and True: simple meristogenous origin of subicle in which a cell swells and branches by budding.

FIG. 127.—Hypha and branches: one branch has developed into a seta.

FIG. 128.—Beginning of symphogenous development of a subicle.

FIG. 129.—Later stage.

FIG. 130.—Many branches interweaving to form a subicle.

FIG. 131.—Complex symphogenously developed subicle.

Epicoccum

FIG. 132.—*Epicoccum*, species indet.: compound meristogenous development of simple sporodochium with young spores.

Meliola(?)

FIG. 133.—Pycnidium with *M.*(?) *camelliae:* 4-cell stage developed from single cell within a hyphal branch.

FIG. 134.—Many-celled stage apparently developed from end cell of hypha.

FIG. 135.—Mature pycnidium developed from single cell within hypha.

FIG. 136.—Same as fig. 135.

FIG. 137.—Slightly different pycnidium from figs. 133, 135.

ECOLOGY OF TILIA AMERICANA

I. COMPARATIVE STUDIES OF THE FOLIAR TRANSPIRING POWER

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 253

JAMES E. CRIBBS

(WITH THIRTEEN FIGURES)

Introduction

Up to the present time transpiration studies have been conducted almost exclusively with potted plants, or upon plants which were growing under controlled conditions. This is due to the fact that most of the investigation has been purely physiological in its aims; and since the factors influencing this process are numerous, they can evidently be more definitely calculated in controlled habitats than in natural ones.

It has been the investigator's aim to carry this line of experimentation into the field, in an endeavor to determine what differences occur in the relative foliar transpiring power of a certain species when growing in different environments. It was at once recognized that the complication of factors which influence transpiration is considerably increased, and the precision with which they may be measured and the relative values to be attributed to each are perhaps less exact than when such investigation is made under greenhouse or laboratory conditions. Nevertheless, there are certain problems which of necessity must be worked out under field conditions, especially when we wish to determine the ecological value or relationship of the environment.

For this investigation *Tilia americana* was chosen because it, perhaps more than any other of our tree species, grows under a wide range of environmental conditions. This is especially true when we consider its unusual ability (as a member of a mesophytic forest) to surmount moving dunes which chance to advance upon it. This ability of *Tilia* to persist on the moving sands brings it

into a set of varying conditions which are widely different from those of its normal mesophytic environment, and affords an excellent opportunity to investigate certain ecological and physiological features which are brought under particular stress because of the abrupt change in growth conditions.

Five stations for investigation were selected on the dunes near Miller, Indiana, and will be designated here as *A*, *B*, *C*, *D*, and *E*.

FIG. 1.—Station *A*, showing *Tilia* on forested complex, associated with *Psedera*, *Smilacina*, *Acer*, and *Prunus*.

Studies were conducted also at station *F* in a mesophytic forest on morainic clays in western Pennsylvania, that a comparison might be had for different soil conditions.

Station *A* (fig. 1) is located on an established dune complex which has advanced to a state of mesophytism in regard to both the tree and herbaceous vegetation. It is well sheltered from the strong lake winds, and is not exposed to the intense light and the accompanying high temperatures of the open sand areas.

Station *A* lies deeper in the complex than *B* (fig. 2), which is situated at the base of a forested dune and is not more than 15 m.

from the edge of a blowout. The conditions are very similar at *A* and *B* so far as the humus is concerned. This has attained a thickness of approximately 5 cm., which is indicative of a relatively long period of stability. The presence of a well defined humus is correlated with a very rich development of herbaceous undergrowth and tree seedlings.

FIG. 2

FIG. 3

FIGS. 2, 3.—Fig. 2, station *B*, showing position of *Tilia* at base of well established dune and with same plant associates; fig. 3, station *C*, where *Tilia* is located on edge of blowout; humus being broken up by sand movement, and access of wind and light much greater than at *A* or *B*.

Station *C* (fig. 3) is located on a west-facing slope on the edge of a blowout where there is a ready access to strong winds. The exposure to light is much greater here than at either *A* or *B*, especially in the afternoon when the sun's rays fall vertically upon the slope, while the stations in the forested complex are deeply shaded. The free sand movement has destroyed the humus and

with it a large part of the accompanying herbaceous vegetation, thus developing a situation of greater openness and more intense exposure.

Station *D* (fig. 4) is located on the lee slope of an advancing dune. In most respects this position is more xerophytic than that at *C*, for there is a complete absence of humus and herbaceous

FIG. 4

FIG. 5

FIGS. 4, 5.—Fig. 4, station *D*, with *Tilia* on lee slope of advancing dune; sand advance rapid at this point, and absence of humus further inhibits herbaceous development; plant associates: *Cornus stolonifera*, *Ammophila arenaria*, and *Prunus virginiana;* fig. 5, station *E*, showing *Tilia* located on crest of high dune and exposed to most desiccating conditions found in dune environments.

undergrowth. The incipient rays are strongly reflected from the open sand, giving a very high light intensity and considerable increase in temperature. Being on a south-facing slope, the greatest exposure occurs in the late forenoon, followed early in the afternoon by shade which is continuous until evening.

Station *E* (fig. 5) represents the most exposed habitat to be found on the open sands, as it is situated about 25 m. above the

lake on the crest of an eroding dune which is exposed to wind from all points of the compass. The water content is less at this point than at lower levels and the light intensity becomes the greatest, the sun striking the station about 8:00 A.M., from which time it remains directly exposed until evening. The absence of humus and the exposure to wind combine to give a high sand mobility, which means a most unstable kind of habitat.

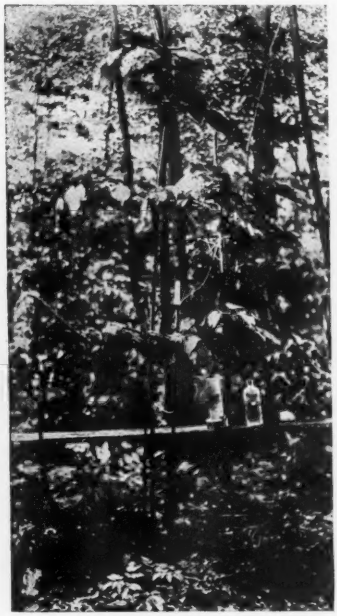

FIG. 6.—Station *F*, showing *Tilia* in mesophytic forest on clay soil; chief plant associates: *Osmorhiza*, *Adiantum*, *Caulophyllum*, *Aralia*, and *Actaea*.

Station *F* (fig. 6) is located in a forest, the chief tree members of which are *Acer*, *Liriodendron*, *Castanea*, and *Prunus serotina*. The herbaceous undergrowth includes such mesophytic species as *Aralia racemosa*, *Adiantum pedatum*, *Osmorhiza longistylis*, *Viola pubescens*, etc. The humus at this station has a depth of about 5 cm. and the underlying soil is a mixed morainic drift.

Methods

The cobalt chloride paper method was employed to determine the relative transpiring power. This method, first used by STAHL (15), has been improved and employed by subsequent investigators and is undoubtedly the one in present use which is best adapted for work in the field. Whatman's filter paper no. 30 was used throughout this work and was treated with a 3 per cent solution of cobalt chloride and prepared in accordance with the method described by LIVINGSTON and SHREVE (10). Preliminary tests were made with the plain paper and the tricolor slips of LIVINGSTON and SHREVE, and as essentially the same coefficients

were obtained the tricolor paper was discarded as being more difficult to prepare and less easily handled in the field. The plain paper has the additional advantage of being somewhat slower, and is thus more satisfactory when used at stations on the open sands, for the change in color was found to take place so quickly that even the paper with longer time values was frequently difficult to read. The probability of error arising from short time periods was largely eliminated by increasing the number of readings from the usual 5 to about 8 or 10, and the time recorded in each instance was the average of all readings made.

The paper was applied to the leaf surface by means of the clip devised and described by LIVINGSTON (8). At each of the stations leaves were chosen which were about 1 m. above the ground; readings were taken from the same relative position on the different leaves; and so far as possible the same set of leaves was employed in each subsequent day's work. Records were taken from 2 leaves at each station, usually at hourly intervals, beginning as soon as there was sufficient light to observe the color change and continuing until darkness prevented further reading. Records given here were taken from the abaxial (stomatal) side only.

The indirect method of determining the color change over the standard evaporating surface, as suggested by BAKKE (1), was used during the second summer's work, thus making it unnecessary to take the standard cup of LIVINGSTON and SHREVE into the field.

Various devices were tried for heating the hygrometric paper to force off the water of crystallization, for considerable difficulty was experienced because of the prevalence of stong lake winds, so a special lamp was devised and used for this purpose. The chief difficulty with the acetylene lamp was to get a steady flame for a long period of time. That which was finally employed consisted of a railroad lamp, with a round wick, which had an oil capacity of about 0.5 liter. A tubular piece of tin was fashioned so that it had a transverse dimension of about 8 cm. and a length of 23 cm. This was fitted to the base by means of 3 guides so as to leave a space of about 2 mm. below for ventilation. Into the

upper end was set a small tin cup such as may be obtained at any hardware store, and over this a screen lid was placed for use when working in a high wind. For ventilation above the flame, a ring of small holes was cut about 2.5 cm. below the level of the inserted cup. This lamp was found to be readily controlled, burns alcohol or kerosene equally well, can be used successfully in a strong wind, and burns continuously for 48 hours or more without refilling or adjustment.

Relative humidity was calculated hourly by means of the sling psychrometer, such as is in use by the United States Weather Bureau, and the wet bulb depression was referred to a standard table as given by MARVIN (12) to obtain the relative humidity values.

Soil temperatures were recorded for depths of 2 dm. and 4 dm. by means of a centigrade thermometer mounted on a cylindrical piece of hickory which was well adapted for inserting into the sand. Atmospheric temperature records were taken and recorded hourly during each day of experimentation.

Soil samples were collected at the different stations on the days when these were worked and the moisture computed on the basis of dry weight. From these results growth water was calculated by the equation GW = SW − WC, in which SW = total soil water and WC = the wilting coefficient. Samples were taken from depths of 2 and 3 dm. and dried for 6 days at a temperature of 100° C.

The wilting coefficient of the soil was computed by the centrifuge method of BRIGGS and MCLANE (4). The moisture equivalent was obtained directly and the wilting coefficient derived from this by the equation of BRIGGS and SHANTZ (3), $\frac{ME}{1.78} = WC$.

The evaporating power of the air was recorded by means of the porous clay cup of LIVINGSTON (7). Hourly readings were readily secured by mounting the atmometer on a graduated burette tube which was refilled when the water column fell to a point about 25 cm. below the level of the cup.

Field work was conducted under diverse weather conditions to discover to what extent the varying of such factors as relative

humidity, wind, light, temperature, etc., might influence the rate of transpiration in the field, and to compare its relative influence in the different environments. In each instance readings were taken at stations *A*, *B*, and *C* on the same day. The same may be said for stations *D* and *E*. Fig. 7 represents the records of relative transpiring power, temperature, relative humidity, and evaporation for *A*, *B*, and *C* on July 21, 1918. As in the following graphs, the scale to the left is for the index of foliar transpiring

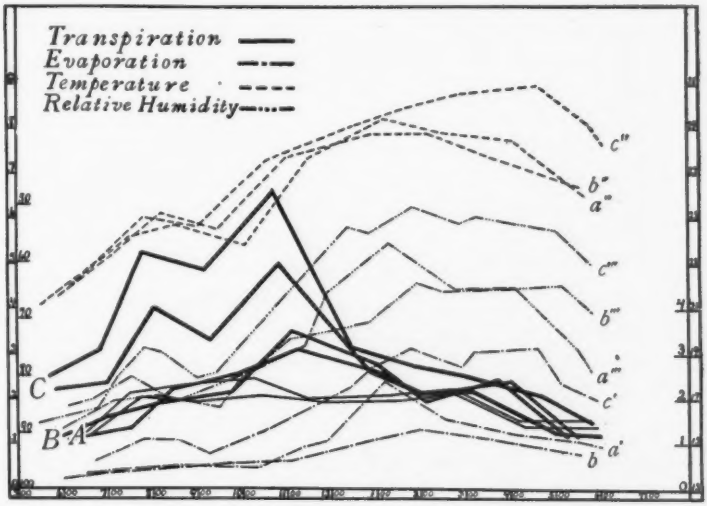

FIG. 7.—Graph giving transpiration, temperature, evaporation, and relative humidity curves of stations *A*, *B*, and *C* for July 21, 1918; scale similar in all following graphs (see text).

power; the inner one to the left is for relative humidity; the inner one to the right is for evaporating power of the air and is expressed in cubic centimeters per hour; and the scale to the right records the atmospheric temperature in degrees centigrade. Time is indicated at the bottom of each graph.

It will be noted that there is a close parallelism between the curves representing temperature, evaporation, and relative humidity. This is in fact what would be expected, as these factors are

closely interrelated. Accompanying the opening of the stomata in the morning, there is a rise in the transpiration indices, and their curves follow closely those of the factors just mentioned until about 11:00 A.M., when a marked divergence occurs. The transpiration curve declines rapidly from a maximum at this time, while the curves representing relative humidity, temperature, and evaporation continue to rise until about 2:00 P.M., following which there is a gradual decline. This feature is little in evidence in the graph of station *A*, but becomes more so in those of *B* and *C;* that is, the divergence becomes more and more conspicuous with an increase in exposure of habitat. This same feature will be found to recur with greater prominence when we consider the data of stations *D* and *E* on the open sand.

There has been considerable discussion concerning the interpretation of this sudden decline: LLOYD (11), following his investigation of stomatal action in *Fouquieria* and *Verbena,* concluded that the earlier view that stomatal movement controlled the transpiration rate was not entirely true, for there may be an increase in the transpirational loss for some time after the maximum opening; and there was found to be frequent decrease and subsequent increase in the afternoon, with little or no accompanying stomatal movement. RENNER (13) advanced the concept of "saturation deficit" to explain this behavior in transpiration, and LIVINGSTON and BROWN (9) in the following year discussed this behavior under the "incipient drying" theory. These are essentially the same concept, namely, that following the rise in the transpiration rate which accompanies the stomatal opening in the morning a point may be reached when the turgidity of the mesophyll cells of the leaf becomes sufficiently reduced to increase the surface tension of the water films in the walls. A check in the molecular diffusion of water from the cells follows. The increased concentration of the cell contents would then exert additional pull on the water in the translocating system, and a restoration of turgor may then follow without visible wilting occurring in the leaf. The length of time elapsing before the restoration of turgor depends largely upon the evaporating power and temperature of the atmosphere, also upon the relative humid-

ity of the air, and in some cases upon growth water. In recording the daily transpiration march in *Tilia* this saturation deficit was found to be very characteristic, especially at times when the relative humidity is low. It is also induced more readily when the growth water is near the zero point.

There is another condition, however, under which a very similar behavior in the graph is noted and may very easily be confused with the preceding. This would be especially liable to happen when one is employing the porometer and cobalt chloride paper only, as was done in the work of TRELEASE and LIVINGSTON (16). This cause lies in a sudden change in relative humidity. Thus if there is a sudden increase in relative humidity the effect on the transpiration index is to depress it. Such depressions may be detected from saturation deficits by an accompanying lowering in the curve of evaporation, for when it is due to a deficit the evaporation curve will be high. Inasmuch as a depression in transpiration caused by an increase in relative humidity is not accompanied by a closure of stomata, according to LLOYD (11), the porometer of course would continue to record the relative stomatal opening, and a divergence in the graphs would result. Instances of this appeared a number of times during the experiments, and were very marked on the occurrence of transient showers.

The sudden depression at 8:00 A.M. in graphs of station *C* (fig. 7) is due to this influence. It will be noticed that there is a corresponding increase in relative humidity, with a lowering of temperature and evaporation power. The behavior at this time is definitely attributed to the passing of a thundershower some distance to the south. It is interesting to note that the influence is more pronounced at station *C* than at *A* or *B*, especially as regards transpiration and evaporation. The drop in evaporation at *C* is attributed mostly to a sudden period of calm which had less effect at *A* and *B* because of their more sheltered position. The greater effect in the relative transpiration curve at *C* is probably to be interpreted as the result of a greater susceptibility of the leaf to a change in relative humidity when transpiring at a high rate.

The curves in fig. 8 were plotted from readings taken at station *D* on September 2. One of the outstanding features of this graph is the parallelism in all the curves. It will be observed that the maxima of transpiration coincide with those of temperature, evaporation, and relative humidity; and that there is an absence, or at least almost a complete absence, of an afternoon saturation deficit. In both of these respects this graph differs from the preceding one. The difference was found to be related to the atmospheric conditions. The evaporating power of the air

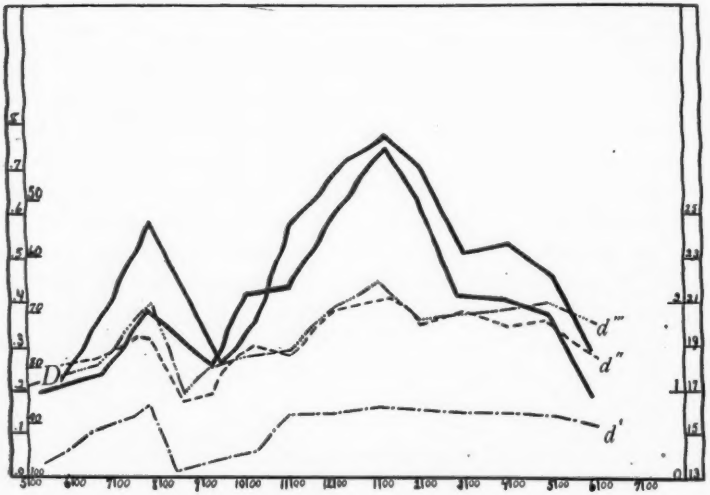

FIG. 8.—Graph plotted from data taken at station *D* on September 2, 1918; parallelism of curves and absence of saturation deficit due to high relative humidity.

was low throughout the day, never reaching 1 cc. per hour, while ordinarily at station *B* it became 2 cc. or more per hour by 2:00 P.M.; the temperature was low and the humidity high. This combination of factors in the field was always found to favor parallelism of curves and an absence of a saturation deficit. Under such conditions the maxima usually occur later in the day than when the deficit is developed.

At 7:30 A.M. there occurs a sudden fall in the transpiration indices which resembles the deficit drop. This was due to a sud-

den shower which began at 7:40 and lasted until 8:20. If the stomatal behavior is similar in *Tilia* to that found by LLOYD (11) to exist in *Fouquieria, Verbena,* and *Ampelopsis,* a porometer curve, if such had been made, would probably have continued to rise from 8:00 to 10:00 A.M. and would have given the type of curve that is obtained when a true deficit occurs. The evaporation curve alone shows that this was not a deficit depression, and one could safely infer the same from the much higher maximum that immediately follows; but if such a drop had occurred near midday, the second higher mode may not have occurred and a fall identical with that of the saturation deficit may have resulted. It will be noted that, notwithstanding the low evaporation rate, the transpiration index is quite high. This was found to be true for positions on the open sand, although under the same atmospheric conditions on humus the index was always much lower. This is probably largely due to the greater light intensity in the former position, and to a certain degree to higher temperatures.

It may be interesting to note the following atmospheric conditions in their general relation to the transpiration indices for this particular day: 5:00–7:20 A.M., partly cloudy; 7:20–7:40, cloudy; 7:40–8:20, rain; 8:20–9:30, cloudy; 9:30–10:00, clearing; 10:00–12:30 P.M., sun; 12:30–3:00 P.M., cloudy; 3:00–6:00 P.M., clear, but station shaded. From these data it will be seen that the drop from 1:00 to 3:00 P.M. was probably due to the sudden cloudiness of that period, which may have caused sufficient closing of the stomata to effect a depression. There is also a small drop in temperature and relative humidity at this time which would also have their influence, although not recorded by the atmometer. It should be said, however, that the white cup is less influenced by light changes than is the leaf (LIVINGSTON 6), and this was found to be most noticeable on the open sand when the index of transpiration was high.

One of the most striking relations that appeared in these comparative studies was found in the readings taken at the stations located on humus and those on the open sand during the latter part of the summer. As previously stated, stations *A* and *B* are in a forested complex which has a well developed humus

and an abundant herbaceous undergrowth, and *C* is on the edge of the same complex, where it is being destroyed by a blowout. These stations, when compared with *D* and *E*, stand out in contrast by their greater mesophytism. This is true in regard to the texture of their foliage and its richness; but during the months of August and September the *Tilia* complex rapidly undergoes a change which is very noticeable in the vegetation and is conspicuous in the relative transpiration indices. This change is initiated by an early reduction of the soil moisture to the wilting coefficient, evidently because of the heavy vegetation the sands are supporting and the excessive transpiration rates caused during this period by the highly desiccating atmospheric conditions. Stations *D* and *E* show, during this same period, a higher growth water content on the exposed dunes; and the abscission which is carried on rapidly in the *Tilia* complex is entirely unnoticed here until much later. The greater soil moisture on the open sand as compared with the pine and oak dune stages has been pointed out in the work of FULLER (5), and is seen to be similar for the *Tilia* complex. Fig. 9 emphasizes this relationship. The data for stations *A*, *B*, and *C* were taken on August 26, and that for station *E* on August 11. Although the 2 sets of readings were not taken at the same time, the atmospheric conditions on the 2 days were practically identical. Both days were sunny throughout, and the general parallelism of temperature, evaporation, and relative humidity was rather unusual. Curves for these factors were plotted for stations *A* and *E* only.

The transpiration indices of *A*, *B*, and *C* were all very low, that of *C* being slightly higher than *A* or *B*. They rose slowly in the morning from a low point and reached a low maximum at 8:00–8:30 A.M. A deficit occurred then because the soil moisture had reached the wilting coefficient, and the indices remained low throughout the day, rising slowly in the evening as the leaves regained their turgor. Visible wilting occurred about 10:30 A.M. at these stations, and was sufficient to cause stomatal closure. The temperature, evaporation, and relative humidity are seen to have remained high, reaching a maximum about 3:00 P.M., after which they declined rapidly.

A very different behavior occurred at station *E*, where the exposure is more intense. The growth water here was found to be 0.679 per cent at 2 dm. and 1.054 per cent at 3 dm., but the leaves remained turgid throughout the day, and there was no visible wilting. The transpiration index at 6:00 A.M. was quite high, and rose rapidly during the morning to a maximum at 12:00 noon. Here a saturation deficit was developed and a sudden

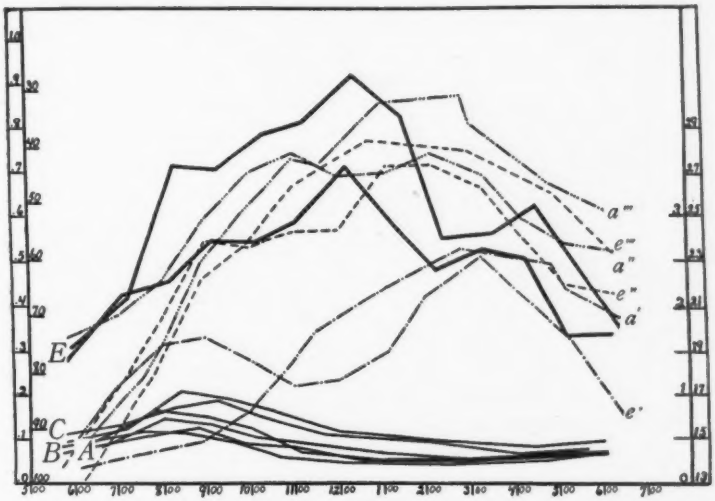

FIG. 9.—Graphs for stations *A*, *B*, and *C* on August 26 and station *E* on August 11, 1918; high transpiration index occurs on open sand with afternoon saturation deficit, while indices for humus stations remain low, due to visible wilting.

depression occurred until about 3:00 P.M., which was followed by a second low mode at 4:30.

This curve is a very typical one for a clear day at this position, in that there is a rapid rise in the morning to a high maximum, and a following clearly marked depression due to a saturation deficit, but no visible wilting occurs; then a second low mode in the afternoon about 4:00 P.M., followed by a rapid decline with the closing of the stomata in the evening. The morning maximum occurs from 9:00 to 12:00, unless disturbed in some way by

such influences as thundershowers and sudden shifting of wind to or from the lake with accompanying atmospheric changes in temperature, relative humidity, etc.

The usual pronounced saturation deficit that occurs on the exposed sands is shown in fig. 10, which is a graph of readings taken at station *E* on July 27. There was a very rapid rise to a maximum at 9:30 A.M., at which time the water loss presumably

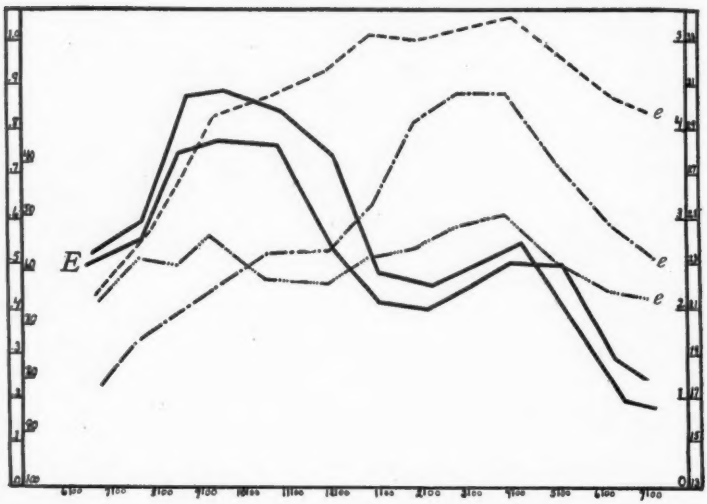

FIG. 10.—Graph plotted for station *E* on July 27, showing typical saturation deficit developed in open dune environments on clear days with temperature high or relative humidity low.

equaled the maximum translocating ability of the plant under the conditions. Then as a deficiency was created in the cells by an excessive loss, a drop in the index occurred until the turgor was regained. A second low mode occurred at 4:00 P.M., a feature recurring in all records taken at stations *D* and *E*, in which an appreciable deficit was developed. Although the decline in the relative transpiration index was considerable in the early afternoon, there was no visible wilting.

Fig. 11 is a composite graph in which the curve for each station is plotted from the average of all the readings taken. This figure shows the relative transpiring power of *Tilia* in the different dune environments, and it will be seen that there is a very pronounced increase in the index of transpiration when considering the stations in their order from the mesophytic to the more xerophytic habitats.

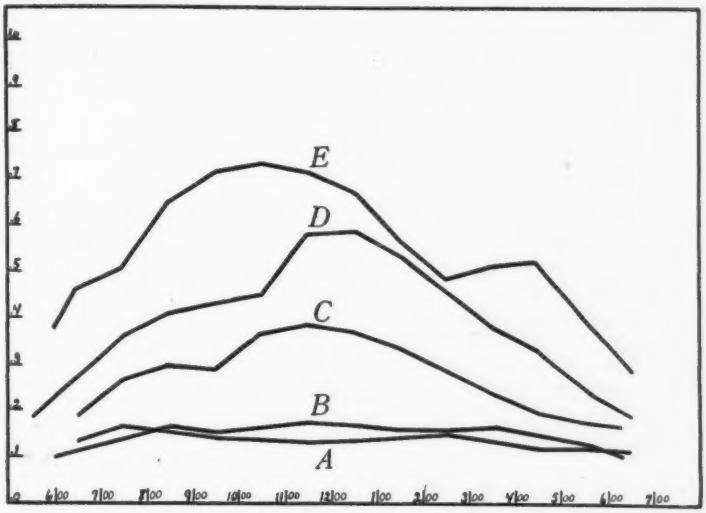

Fig. 11.—Composite graph of all transpiration readings taken at stations *A*, *B*, *C*, *D*, and *E*, showing increase in transpiring power accompanying increased exposure of habitat.

These curves being averages, and hence not recording daily variations, do not give to best advantage the typical daily curve. The occurrence of the maximum about 12:00 noon in the more mesophytic situations, however, and an earlier occurrence from 9:00 to 11:00 A.M. on the open sands, is noticeable even in the average graphs, as is also the earlier morning rise characteristic of the exposed stations.

Fig. 12 includes averages of all the different factors taken in connection with the dune transpiration studies. The unit spaces at the top of the graph have the following values: evaporation

5 cc. per unit; transpiration 0.1; relative humidity 15 per cent; soil temperature 7° C.; atmospheric temperature 10 per cent; and growth water 1 per cent per unit. From these it will be seen that the conditions existing at stations *A* and *B* are closely similar; but from *B* to *E* the stations represent a graded series of habitats as regards these factors, just as clearly as they do when we consider their comparative positions in the vegetative cycle. This graded variation found in the dune environments is much more pronounced than that for different situations on clays, which will

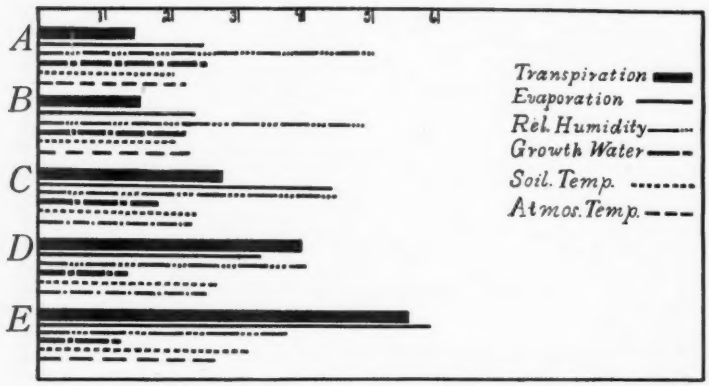

FIG. 12.—Comparative graph illustrating conditions of transpiration, etc., for the 5 dune environments; close gradation of factors evident here; increase in transpiration accompanying decrease in growth water is characteristic of dune habitats.

be mentioned only briefly in this paper. The increase in relative transpiring power that occurs with an increase in exposure of habitat is very evident.

Concerning evaporation, the only variation occurs at station *C*. Here the water loss is considerably more than at *D*. This is because of the greater access of wind at *C*, which is located on the edge of a blowout, while *D* is on a protected lee slope where only south and southeast winds have access.

The average relative humidity decreases rapidly from *A* to *E*, and is almost parallel with the increase in the transpiration index. Variation in humidity has been considered the most poten-

tial single factor influencing the transpiration index (BRIGGS and SHANTZ 2), and so far as considered in these studies it has been found to be a factor of the first order. It is not always the controlling one, however, for there are frequent variations in the daily march which owe their appearance to other causes. Temperature, stomatal movement, and growth water, for instance, at any given time may become the dominating factor, only to be succeeded as the conditions vary by some other which for the time being replaces it.

The proportional increase in the soil temperature over that of atmospheric temperature from *A* to *E* is of interest, and it may safely be inferred that high soil temperatures have a prominent part in the maintenance of the water supply, and hence indirectly concern the transpiration rate. Until more accurate quantitative measurements are made upon the effect of increase of temperature of the soil upon the water absorbing power of roots, however, the quantitative influence of this factor can only be speculated. Such work as has been done by RYSSELBERGHE (14) on the effect of temperature changes on the rate of osmotic transfusion through semipermeable membranes leads to the conclusion that the much higher soil temperature, as of *E* over station *A*, must be one of the factors which enable the exposed plants to take over water from the soil with sufficient rapidity to withstand the much higher transpiration loss.

One of the noteworthy features of the dune studies appears in the relation of the growth water to transpiring power. It may be seen from the figure that there is a low average of growth water at all stations, that for *A* being only about 2.46 per cent and that for *E* 1.25 per cent. While it is true that the highest average is at *A*, the greater percentage here is due to the higher content during the earlier summer months. During the latter part of July and August, however, the water available for plant absorption decreases more rapidly than on the open sand, and the frequent drop in soil moisture to the wilting coefficient produces a period of stress and leads to early abscission. Meanwhile the growth water on the moving complex remains practically constant, especially at a depth of 3 dm. or below, where it is about

1.25 per cent or more. This greater constancy is attributed to the moving mulch of dry sand which breaks the continuity of surface films in the soil particles, thus preventing rapid evaporation. This ability of a plant to maintain a higher transpiration index with a growth water content of 1.25 per cent than the same species does with 20 per cent or higher, such as is commonly true for clays, indicates that the amount of growth water has but little relation to the transpiration index so long as the soil moisture content remains above the wilting coefficient. This gives a somewhat unusual aspect to the question of mesophytism and the part played by soil water as a factor in plant growth. So far as *Tilia* is concerned, it produces more vigorous vegetative structures, which retain their activities later in the summer, when growing in open situations on sand than when in the forested dune complex; and the factor of greatest importance seems to be, not the average growth water of the soil, but whether the available moisture repeatedly falls below the wilting coefficient. So long as it is above that point, although it is by only a very small percentage, the normal activities, including high transpiration, are carried on.

There is one point concerning this relation which needs investigation, however, namely, the relative extensiveness of the root systems in the two situations. I am inclined to the idea that the ability of *Tilia* to develop adventitious roots when covered by an advancing dune may enable such individuals to draw their water supply through a root system the absorptive surface of which is greater in proportion to the amount of foliage than is true of this species when growing in the forest complex. Another point of probable difference is that the individuals on the open sand may obtain a considerable portion of their water from a greater depth than do those on the humus; but the extent to which the root systems persist when submerged by advancing dunes has never been worked out.

As indicated by the averages shown in fig. 11, the situation at *E* is distinctly more xerophytic than at *A*. Every fact recorded in the experimentation points to this conclusion. The size,

general shape, and texture of the leaves at station *E* are also distinctly xerophytic, but it has been noted that notwithstanding this the transpiration index is higher, a fact not at all in accordance with the behavior of desert plants so far reported, for they are characterized by a low transpiration index, as pointed out by several workers. With this fact in view BAKKE (1) has suggested the foliar index of relative transpiring power as a test for the mesophytism of a plant, as follows: "As a result of the preliminary study, it may be suggested that plants exhibiting a diurnal foliar transpiring power of less than 0.30 may be regarded as xerophytes, while those exhibiting indices above 0.70 may be considered mesophytes." It may be seen at once that the behavior in *Tilia* is the reverse of that common to desert plants, and hence an application of this test would lead to confusion. I believe with BAKKE, however, that with certain reserve this method may be used as a fair indicator of mesophytism, provided two precautionary measures are taken: first, that the species under consideration be chosen in its normal environment and not in an abnormal or forced one; and second, that hourly readings be taken for at least two full days and that the relative humidity and temperature conditions be carefully employed in calculating the results. This latter precaution is necessary because of the great variation in the index at different times of the day, in the first place, and because of the wide variation of the indices at any particular hour on two successive days when the relative humidity or temperature has undergone considerable change.

The development of a xerophytic leaf under unusual conditions of exposure has been found in *Tilia* to result in a leaf less effective in preventing water loss than are desert types. This may be attributed to a lagging of the effect behind the causal factors. On the other hand, such lagging may be considered as not occurring, for there is always a favorable balance in water relations which permits a greater vegetative activity than would be possible in desert plants, and becomes possible on the open sands only because of a sufficient and permanent growth water throughout the growing season.

At station *F* there is considerable difference in the environment, and the factors accompanying it, from that of the dune series. It has already been noted from the introductory description that the undergrowth is composed of a more shade-requiring assemblage than even the most mesophytic positions found on the established dune complex. The humus is slightly more developed, but the soil underlying it is very different, and unlike in the forested dune habitats the growth water was always adequate to support an abundant and diversified vegetation, and never reached the wilting coefficient. The average available water at 2 dm. was 19.40 per cent and the minimum 12.45 per cent. Thus if growth water is inducive of a high transpiration, one would expect to find it here, but the average transpiration at this station for 5 complete days' readings was only 0.16, an index practically identical with that of station *A* of the *Tilia* forested complex where the growth water averaged 2.5 per cent.

The same tendency displayed by the dune graphs to show curves with a single mode recurs here where the mesophytism is greater. The maximum power of transpiration comes later in the day, commonly from 12:00 to 2:00 P.M., and is more frequently coincident with the maxima of temperature, evaporation, and relative humidity. This relation is shown in fig. 13, which is a graph of station *F* on June 14. At this time there was a growth water content of 26.74 per cent and a relatively low humidity. The temperature was low, while the evaporation was high when compared with the transpiration, higher than commonly found in dune environments. The morning rise of the transpiration index was very slow and the maximum reached was not very high. On this particular day it was clear until about 2:00 P.M. with increasing cloudiness through the afternoon, followed by showers at 8:00 P.M. Although it was clear during the forenoon the station was shaded throughout the period.

The fact that direct sun upon the leaves in a habitat of this sort almost always leads to a rapid increase in the transpiration rate would suggest that the low light intensity of the densely shaded forest is one of the chief factors leading to the low average commonly found there.

Summary

1. Cobalt chloride standardized paper was found well suited for comparative studies in the relative transpiring power of leaves in the field.

2. The daily march of transpiration in *Tilia* was found to vary greatly for the same leaf on different days. This variation was found to be influenced by relative humidity, temperature, light intensity, soil moisture, and presumably by soil temperature.

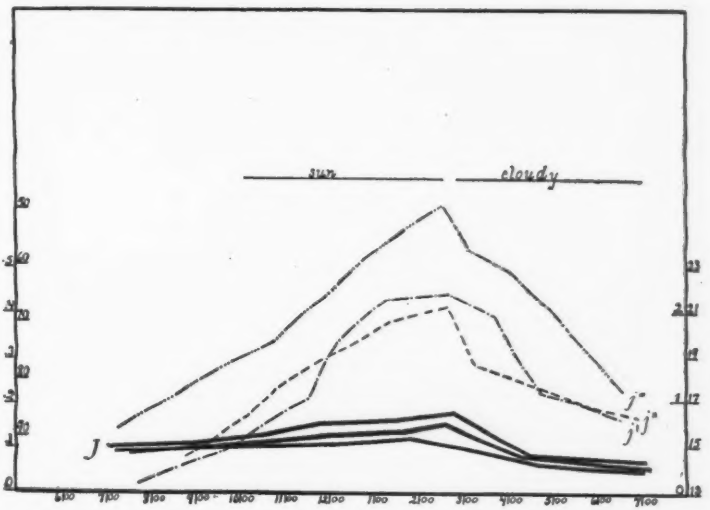

FIG. 13.—Typical graph illustrating low transpiration indices occurring in morainic mesophytic forest where growth water is always high; note low transpiration for a day with so low a relative humidity.

3. The foliar transpiring power of *Tilia* was found to increase in dune environments from an index of 0.15 on the forested *Tilia* complex to 0.55 in the most exposed situations on the open sand. In habitats between these extremes the transpiration power was found to be directly proportional to the relative exposure.

4. The morning rise in the daily march is more rapid on the open sand, where it reaches a maximum 1 to 2 hours earlier than in forested situations.

5. In the forested complex the curve representing relative transpiration tends to develop a single mode about midday, and this maximum tends to coincide with the maxima of temperature, relative humidity, and evaporating power of the air. The greater the mesophytism and relative humidity the more striking becomes this tendency.

6. In the most exposed situations on the open sand the relative transpiration maximum usually appears about 10:00 A.M., while the maximum temperature, relative humidity, and evaporating power occur from 2:00 to 4:00 P.M. This divergence from parallelism is due to the development of a saturation deficit, which appears successively earlier as the exposure of the habitat increases. The more mesophytic the habitat the less noticeable becomes this deficit, until it disappears entirely, especially on humid days.

7. The foliar transpiration index is influenced less by wind currents than is the porous cup atmometer.

8. Transpiration curves showing a saturation deficit depression usually develop a second mode about 4:00 P.M., which is, so far as noticed, always lower than the mode preceding the deficit depression.

9. Bimodal transpiration curves have been found to be due either to a saturation deficit or to a sudden increase in relative humidity, although lesser depressions may result from fluctuating temperature or intensity of light.

10. No evidence of visible wilting occurred in *Tilia* on the open sand at any time during the summer, although the so-called "incipient drying" was a common feature of the stations throughout this period. On the forested complex, however, visible wilting occurred during the first week in August because the vegetation was so dense that the water content of the soil was reduced to the wilting coefficient quite early.

11. The average mesophytism on the *Tilia* complex is considerably greater than on the open sand, the growth water averages being 2.5 and 1.25 per cent respectively; but the open positions are practically constant in their water relations, while the forested complex represents a decreasing mesophytism as the summer

advances, the growth water in the spring being higher than at any other time.

12. The amount of growth water in the soil apparently has very little influence on the transpiration index, unless it is reduced to the wilting coefficient. It might be argued that a low growth water is the cause of the saturation deficit depression, but there is evidence that it is due rather to the inability of the translocating system to conduct water to the leaves with sufficient rapidity to offset the transpiration loss, and not to a slowing up of the absorption rate. This is substantiated by the occurrence of the typical deficit in readings on *Tilia* when the growth water was greater than 20 per cent. The drop that occurs when the soil moisture falls to the wilting coefficient is more permanent and is due to stomatal movement which accompanies visible wilting.

I wish to express my grateful appreciation of the encouragement and suggestions given by Dr. GEO. D. FULLER, of the University of Chicago.

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REPEATED ZOOSPORE EMERGENCE IN DICTYUCHUS[1]

WILLIAM H. WESTON

(WITH PLATE XXIII AND ONE FIGURE)

Dictyuchus, one of the less known genera of the Saprolegniaceae, was established in 1869 by LEITGEB (6) to include a single species, *D. monosporus;* and in 1872 LINDSTEDT (7) added the two species *D. polysporus* and *D. Magnusii;* while in 1893 a fourth species, *D. carpophorus*, was described by ZOPF (12). LEITGEB observed that in his type species laterally biciliate zoospores emerged from sporangiospores which were invariably retained *in situ*, and that these zoospores swarmed but once; and since this condition has been found in all the other species, it has been regarded as characteristic of the genus.

In this paper certain observations on an undetermined species of *Dictyuchus* are presented, which show that, in this instance at least, the usual history, as described by LEITGEB and others, may be modified through the presence of a second swarming of laterally biciliate zoospores, which occurs after the first has been completed; and since, so far as the writer is aware, a diplanetic condition of this type has not hitherto been noticed in any of the Saprolegniaceae, a record of its occurrence has seemed desirable, even before a comprehensive study of the fungus has been completed.

The *Dictyuchus* in question appeared in a culture of moist sand, leaves, and other débris taken from a shaded brook bed in a ravine near Great Barrington, Massachusetts; and for over a year and a half it has been kept under observation both in gross and pure cultures, which have been subjected to a variety of cultural conditions. During this period, however, all attempts to induce the formation of sexual organs have been without result, although sporangia were readily and abundantly produced, and it is thus impossible to reach any definite conclusion as to its specific identity. That it cannot be referred to *D. carpophorus* Zopf is evident

[1] Contribution from the Cryptogamic Laboratories of Harvard University, no. 85.

from the quite different form of its sporangia, as well as from the absence of the peculiar tubercles which, according to ZOPF, are characteristic of that form. On the other hand, it is probable, as has been suggested by VON MINDEN (8) and FISCHER (4), that the sexual organs which LINDSTEDT found associated with his *D. polyspora*, and on which he based the species, belonged in reality to a member of some other genus which had accidentally been introduced into his cultures. For this reason the species is perhaps best regarded as in all probability invalid; and there thus remain but two others, *D. Magnusii* Lindst. and *D. monosporus* Leitg., with which the present form may be compared. On a basis of sporangial characters alone it might readily be referred to either of these species, and its failure to produce sexual organs may be due to the fact that it is the antheridial strain of a unisexual (dioecious) type, similar to that which both of the last mentioned species are said to illustrate. On the other hand, it may prove to be a neutral strain, comparable with PIETERS' (9) "*Saprolegnia* no. 66" and the undetermined species of *Achlya* studied by the author (11), having lost its ability to reproduce sexually, at least under ordinary conditions. That this may be the correct explanation is further suggested by the fact that such neutral or non-sexual conditions of *Dictyuchus* have previously been reported by HUMPHREY (5), TIESENHAUSEN (10), and others; while VON MINDEN even definitely identifies a form of this nature with *D. monosporus* Leitg. Although it is quite possible that a similar disposition of the present species might prove to be the correct one, a definite specific reference does not seem justified at the present time.

In order to follow its development in detail, cultures which were known to be uncontaminated by other forms were washed repeatedly in sterile water and placed in a drop on a slide. As soon as the zoospores had emerged, swarmed, and come to rest, they were picked up with a capillary pipette, placed in a few cubic centimeters of sterile water, and sprayed by means of an atomizer on nutrient media contained in Petri dishes. The latter were then examined under a low magnification, the positions of single isolated spores were marked, and, after two or three days'

growth, transfers to stock cultures were made from such mycelia as proved to be uncontaminated. The development of the fungus thus isolated was studied for over a year in Van Tieghem cells, Petri dishes, and battery jars, under a great variety of cultural conditions.

The mycelium in its morphological characteristics is very similar to that of other species of Saprolegniaceae which have been grown in pure culture. Physiologically, however, it is characterized by weak growth, and in consequence requires more frequent transfer and more concentrated nutriment for successful maintenance. The process of sporangium formation in its early stages closely resembles that which is usually found in other members of the family. In the young sporangium initials, filled with dense protoplasm, hyaline clefts arise, extend, and divide the contents into subequal, polygonal spore initials. The sudden shrinking of the sporangium with a concomitant vacuolation of the spore initials now takes place; but the vacuolate condition is more persistent than in other genera, since one or two vacuoles are often retained in the spores at maturity.

In the succeeding stages of its development, however, the sporangium shows itself to be quite different from any other member of the family, save perhaps that of the doubtful genus *Aplanes*. During the swelling of the individual sporangiospores which marks the final stage of development of the sporangium, it becomes apparent that the delicate membrane surrounding each spore has become firmly united, not only to the walls of the adjacent spores, but also to the inner surface of the sporangial membrane. This close union of the walls appears to be a fundamental peculiarity in sporangia of the generic type, and sharply distinguishes it from the abnormal but superficially similar conditions which are occasionally encountered in *Saprolegnia*, *Achlya*, and *Thraustotheca*, when large numbers of spores have failed for some reason to make their escape. We are probably justified, moreover, in accepting HUMPHREY'S (**5**, p. 81) tentative suggestion that this characteristic obtains throughout the genus. Study of the empty sporangium alone (fig. 1) might lead one to interpret its so-called "cell-net" structure as the result of a simple division

into cells, rather than of a process of progressive cleavage; but continuous observation of the successive stages of spore formation affords no evidence in support of this assumption. Moreover, in starved sporangia only partially filled with protoplasm the spores when formed are more separate, and on swelling do not become closely pressed together. Consequently, only an incomplete union of the walls takes place (fig. 2); and the spores become rounded off to a greater degree, so that the resultant structure is more easily understood than the "cell-net" condition in the densely filled sporangia. It is of interest to note that ZOPF (**12**, *pl. 3, fig. 11*) also figures a similar condition in *D. carpophorus*. This union of the walls in the sporangium increases the mechanical strength of the structure; and in consequence the final swelling of the spores is resisted, and they do not burst out of the sporangium, even though it becomes swollen and bulged. Frequently, however, the swelling of the spores, combined with the outward bulging of the terminal wall of the sporangiophore, is sufficient to rupture the sporangial wall at the base (fig. 19), and the sporangium is abjointed as a whole. This occurs quite commonly even in vigorous cultures, as VON MINDEN (**8**) has observed, and does not appear to be the result of degeneration and senescence, as LEITGEB (**6**) and FISCHER (**4**) have stated.

Renewal of the sporangia is effected by cymose branching (fig. 2), and by their formation in basipetal succession (fig. 1). The first method has been regarded as characteristic of *D. monosporus*, and the second as typical of *D. Magnusii;* but the regular occurrence of both methods in our form and even in *D. Magnusii*, according to VON MINDEN, would indicate that these characters are not specific.

In their subsequent development the sporangiospores within the indehiscent *Dictyuchus* sporangium may either emit zoospores or give rise to hyphae of germination. Since LEITGEB'S original description of these processes is quite detailed, only additional or significant points need be mentioned here. The emergence of the zoospore from the sporangiospore, although described at length by this author, is only scantily figured. The accompanying drawings (figs. 3–9), therefore, have been made to illustrate this process.

After the zoospores (figs. 8, 9) have emerged, their general structure and the disposition of the cilia are seen to be very similar to the "secondary" laterally biciliate type in other genera of the family, although these zoospores are longer and more tapering than those of *Achlya* and *Thraustotheca*, while more flattened than those of *Saprolegnia*. On an average the zoospores of our form are about 13 μ long and 10 μ wide. After an active period of variable duration they come to rest, lose their cilia, and encyst, forming spherical, coarsely granular spores which, to avoid confusion, will be called "cystospores" (fig. 10). After a time these may germinate by sending out hyphae in a perfectly normal manner (figs. 16, 17).

When the sporangiospores give rise not to zoospores but to hyphae of germination, the latter push through the enveloping sporangium wall and grow out into the water (fig. 18) in a manner quite similar to that which is said to be characteristic of *Aplanes*. It is to be noted, however, that in *Dictyuchus*, as well as in other genera in which it occurs as an abnormal method of development, such germination only takes place in the presence of nutrient substances, or of such non-nutrient materials as prevent zoospore emergence.

It is clear that the cycle of non-sexual spore formation just described agrees entirely with the usual accounts. In the form under discussion, however, an additional zoospore emergence may take place. This phenomenon was repeatedly observed under the following conditions: A piece of agar covered with mycelium, when transferred from a stock culture to dilute beef extract, rapidly grows to a small compact tuft of hyphae. If this tuft, after a thorough washing to remove the adhering nutriment, is placed in a hanging drop for study, sporangium formation rapidly takes place, and from the sporangiospores large numbers of zoospores emerge and swim about. Finally they come to rest and encyst for the most part along the edge of the drop. After a time some of the encysted spores so situated germinate by hyphae. Many, however, emit zoospores, with the result that all along the edge of the drop may be seen empty cystospores and zoospores

in various stages of emergence. This process, as shown in figs. 10–13, closely resembles the previous emergence of the zoospore from the retained sporangiospores. The zoospore emerging from the cystospore moreover is exactly like the original zoospore which emerged from the sporangiospore. The cultures in which this additional emergence of the zoospore was observed were derived from single spores, and their purity was beyond question.

To appreciate the significance of this repeated zoospore emergence in *Dictyuchus*, it is necessary to consider briefly the corresponding phenomena in certain related types. The genera of the Saprolegniaceae, as is well known, are distinguished by the characteristic peculiarities of their non-sexual reproduction, since a regular and distinct cycle of non-sexual spore production marks each separate genus. It has been customary to arrange the genera in a series in accordance with the degree of simplicity or the complexity of their cycles. Disregarding the question whether such a series represents the elaboration of a simple type or the simplification of a complex one, we may first consider *Saprolegnia*, which has the most extensive cycle. Since in the other main genera the cycles are less extensive in increasing degrees, we may, in accordance with this, arrange a convenient series: *Saprolegnia*, *Achlya*, *Thraustotheca*, *Dictyuchus*, and *Aplanes* (text fig. 1).

The characteristic cycles of non-sexual reproduction in these 5 genera are shown in the accompanying diagrams, which follow the accepted descriptions for all but *Thraustotheca*. In this genus the author has found that the non-motile sporangiospores swell and escape by bursting the enveloping sporangium wall, after which they closely resemble the escaped sporangiospores of *Achlya* in their further development. The genera form a series representing a gradual decrease in the extent of the cycle of non-sexual spore formation. The series ranges from *Saprolegnia*, with its swarming of primary and secondary zoospores, through successive stages to *Aplanes*, which is believed to lack both of these phases of zoospore activity.

In the repeated emergence of its zoospores this species of *Dictyuchus* shows a distinct departure from the cycle of spore formation

that is customarily ascribed to the genus, nor has this phenomenon been reported in the case of other members of the family. In *Pythium*, however, which is more or less closely connected with the Saprolegniaceae, according as one or another of the several theories of relationship is accepted, a similar double swarming of laterally biciliate zoospores has been reported. CORNU (3) in 1872 first noted the fact as follows: "les zoospores dans tous ces genres

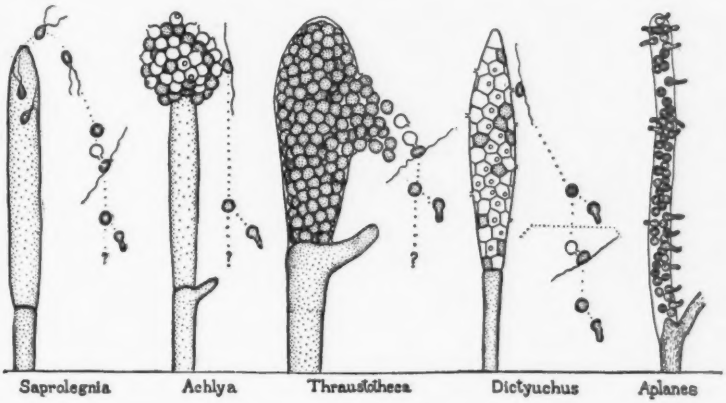

FIG. 1.—Comparative view of cycles of non-sexual spore formation in main genera of Saprolegniaceae; diagrams, of approximately same scale, from drawings of living material, except in case of *Aplanes*, which is after DEBARY'S figure.

germent en donnant lieu à un filament ... ou bien elles émittent des zoospores semblables à elles-mêmes (ex. *Pythium proliferum* et ses var.)." Recently BUTLER (2) has corroborated CORNU'S observations by describing and figuring the process in the case of *Pythium diacarpum*. In spite of the apparent rarity of this phenomenon, however, the writer ventures the opinion that further investigations will bring to light other cases, not only in *Dictyuchus*, but also in related genera of the Saprolegniaceae.

The significance of this repeated zoospore emergence in *Dictyuchus* is a matter of some interest. One may, of course, regard it as a regular but hitherto unobserved stage of the life cycles of

the genus; but in view of the detailed investigations of LEITGEB and others it seems doubtful if such is the case. It must be admitted, however, that *Saprolegnia* had been studied for many years before LEITGEB observed in it the diplanetism on which he based his genus "*Diplanes*." On the other hand, the repeated zoospore emergence in the present instance might be regarded as peculiar to this particular and perhaps hitherto unnoticed species of *Dictyuchus*. Since such an assumption is contrary to our customary conception of the fixity of generic characteristics, its truth may well be doubted. In view of the tardy recognition of the extent of this phenomenon in the genus *Pythium*, it is possible that it has merely been overlooked in the recognized species of *Dictyuchus*. Finally, it is possible that this phenomenon occurs, not only in various species of *Dictyuchus*, but also in other genera of the Saprolegniaceae under certain favorable conditions; and that these conditions either have not been attained in cultures heretofore, or the emergence has escaped observation. It seems highly probable that this is the case, and that in certain Saprolegniaceae there inheres in the protoplasm, even of encysted zoospores of the second type, the ability to form not only germ tubes but also zoospores; and that under proper circumstances the latter may be produced. Probably, then, as ATKINSON (1) has suggested in the case of *Pythium*, this repeated zoospore emergence may best be regarded as a phenomenon of germination, and one which the author believes can be brought about by certain favorable conditions. It is possible that extensive cultural studies of various Saprolegniaceae, with this end in view, will demonstrate that its occurrence is far more widespread than has been suspected. The writer is obliged to admit, however, that he has been unsuccessful in many attempts to induce repeated zoospore emergence in *Achlya* and *Thraustotheca;* but the well known sensitiveness of the Saprolegniaceae to surrounding conditions makes it possible that these failures may have been the result of faulty methods.

In any case, the occurrence of this phenomenon in even a single species of *Dictyuchus* points to the conclusion that the customary application of such terms as "monoplanetic" and "di-

planetic" may be somewhat misleading, and necessitates a modification of our conception of the condition of monoplanetism and diplanetism in the Saprolegniaceae.

Summary

1. In the characteristics of its non-sexual reproduction the fungus which is the subject of this paper shows itself to be a member of the genus *Dictyuchus*. No sexual reproduction was observed, however; hence it cannot be assigned to any of the recognized species.

2. During the formation of spores within the sporangium, the walls of adjacent spores unite with one another and with the enveloping sporangium membrane, to form a polygonally chambered, indehiscent structure. In this respect *Dictyuchus* differs fundamentally from all other Saprolegniaceae, save perhaps the doubtful genus *Aplanes*.

3. The zoospores which emerge from the sporangiospores come to rest and encyst as is customarily described. From these encysted spores in turn, however, laterally biciliate zoospores may emerge. This repeated emergence of laterally biciliate zoospores has not previously been reported in any member of the Saprolegniaceae.

4. It is the opinion of the writer that future study will prove that this phenomenon may occur in other species of *Dictyuchus*, and perhaps even in other members of the family.

In conclusion the writer wishes to express his thanks to Dr. ROLAND THAXTER, under whose kindly supervision these observations were made.

LABORATORY OF CRYPTOGAMIC BOTANY
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EXPLANATION OF PLATE XXIII

The figures were drawn from living material at the level of the stage with the aid of an Abbé camera lucida. The approximate magnification of the combination of lenses used is given in each case, but applies to the original figures, which have been reduced to about two-thirds of their diameter in reproduction.

FIG. 1.—Two successively formed sporangia, the terminal empty, showing "cell-net" structure; second still containing spores; ×550.

FIG. 2.—Sporangium developed from starved hypha, showing incomplete uniting of spore walls; ×550.

FIGS. 3–7.—Stages in emergence of zoospore from sporangiospore; ×1400.

FIG. 8.—Zoospore after liberation; oblique later view; ×1400.

FIG. 9.—Same just starting to swim away; side view; ×1400.

FIG. 10.—Encysted zoospore (cystospore); ×1400.

FIGS. 11–13.—Stages in emergence of zoospore from cystospore; ×1400.

FIG. 14.—Zoospore just after emerging; side view; ×1400.

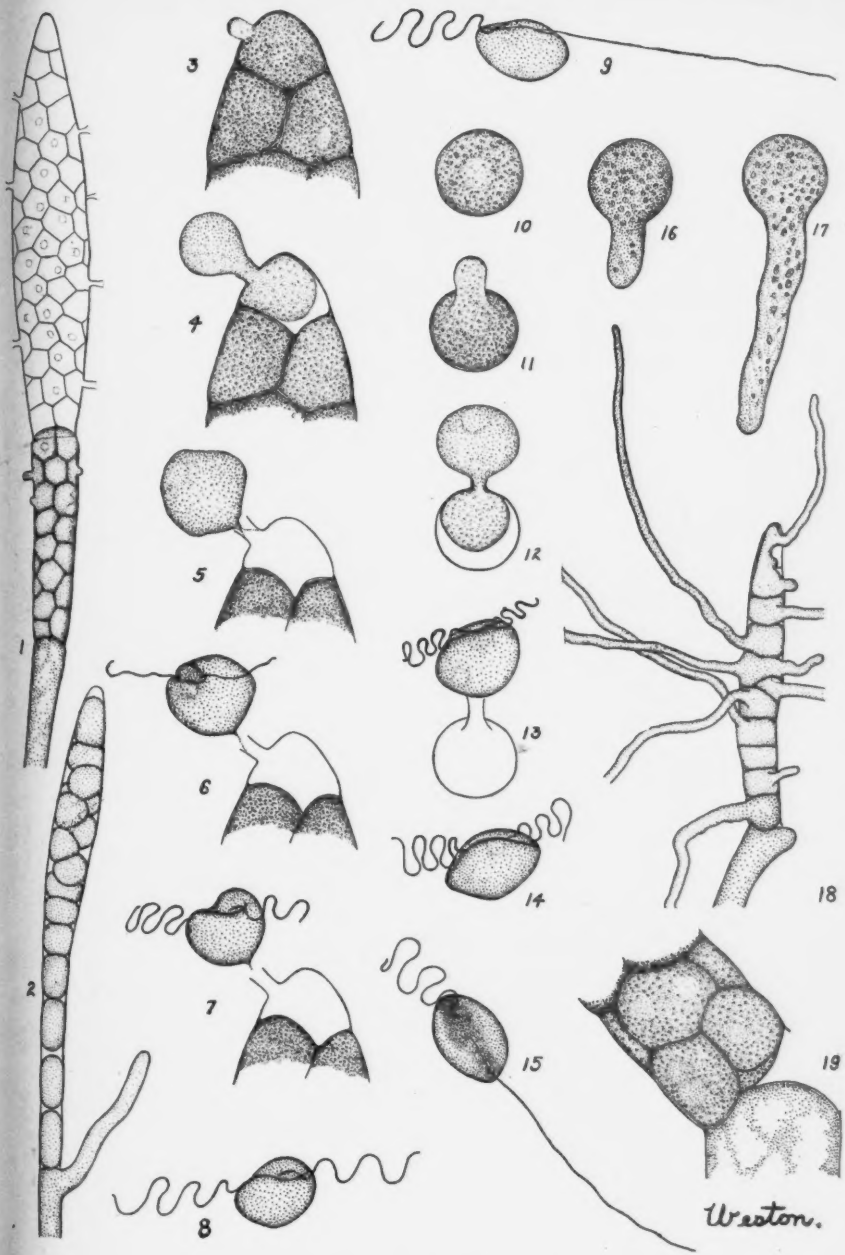
FIG. 15.—Same just starting to swim away; looking down on grooved surface; ×1400.

FIGS. 16–17.—Cystospore germinating by formation of hypha; ×1400.

FIG. 18.—Germination of sporangiospores *in situ* by hyphae; ×550.

FIG. 19.—Separation of sporangium from its hypha by rupture of sporangium wall at base; ×1400.

A



WESTON on DICTYUCHUS

RELATION OF NUTRIENT SOLUTION TO COMPOSITION AND REACTION OF CELL SAP OF BARLEY

D. R. HOAGLAND

In recent years considerable attention has been given to the cell sap from different plants, especially as influenced by varying soil and climatic conditions. Some very interesting general relations have been brought out, but in these experiments it has not been possible to ascertain or control the exact concentration and composition of the soil solution. McCOOL and MILLAR (4), however, have made numerous measurements of the freezing point depressions of the cell sap of both tops and roots of plants growing in soils and nutrient solutions of varying osmotic pressure. These researches have shown clearly that the sap of the plant, particularly of the roots, reflects the concentration of the nutrient solution, whether in the soil or in water cultures.

Comparatively few measurements of the conductivity of the cell sap have been made, although some data concerning this point are quoted by ATKINS (1). The H ion concentration and chemical analysis of the sap have received still less study, yet all these determinations are of the greatest importance in soil fertility investigations. The total osmotic pressure in the plant is known to be dependent to a considerable extent on intensity of photosynthetic action, as well as on the nutrient solution, while the inorganic constituents may well have a more direct relation to the surrounding media.

For a number of years this laboratory has been engaged in the investigation of the relation between the growth of the barley plant and the composition and concentration of the soil solution as shown by analyses of water extracts and freezing point determinations by the method of BOUYOUCOS and McCOOL (2). The work with soils has made it evident that the soil solution is of paramount importance in its effect on crop growth, while, on the other hand, the plant has a marked influence on the concentration and composition of the soil solution. It soon became apparent that the

elucidation of these difficult relationships would require further study by the methods of water and sand culture, which may be subjected to more rigorous control. It is thought that some of the data pertaining to the cell sap of plants from these various soil, water, and sand culture experiments are worthy of a brief discussion at this time.

The soils were kept in large tanks under controlled conditions as described by STEWART (6). The technique of the water and sand cultures will be described elsewhere. The procedure was designed to place, so far as possible, no limitation on the growth of the plant other than the variables under investigation. The nutrient solutions were made to have a composition similar to that of the soil extracts with respect to the important elements. Various concentrations of both acid and neutral reaction were employed. In each concentration acid and neutral solutions had an almost identical osmotic pressure, and the relation between the various ions was very similar. The reactions were governed by the hydrolysis of the various potassium phosphates used.

The procedure employed in obtaining the cell sap consisted in cutting the plant into small pieces, freezing first in brine, then in a carbon dioxide ether bath, and finally pressing out the sap as thoroughly as possible through cheesecloth. It is realized that the exact concentration of the sap is dependent upon the technique employed, but the results are comparative and the general magnitudes, which are of interest in this discussion, are probably not far different from those obtainable with other methods of extraction. Osmotic pressures were determined by the freezing point method, conductivity measurements in the usual manner at a temperature of 25° C. The hydrogen ion concentrations were measured with the aid of the hydrogen electrode, using the apparatus described by SHARP and the author (5). The data for the freezing point depressions, conductivity, and hydrogen ion concentration of the various cultures are summarized in table I.

Effect on osmotic pressures

Considering the total osmotic pressures first, it will be noted that both the tops and roots, either in the acid or neutral solutions, reflect the concentration of the nutrient solution, although no very

definite relationship is apparent. The highest osmotic pressure is found in the sap from the plants grown in the acid nutrient solution of highest concentration (1.72 atmospheres). The roots of these plants showed marked evidence of injury. In all cases

TABLE I

OSMOTIC PRESSURE, CONDUCTIVITY, AND H ION CONCENTRATION OF PLANT SAP IN WATER, SAND, AND SOIL CULTURES

Total solids approximate p.p.m.	Nutrient solution			Plant sap from tops			Plant sap from roots		
	Osmotic pressure (atmospheres)	Specific resistance (ohms)	H ion concentration P_H	Osmotic pressure (atmospheres)	Specific resistance (ohms)	H ion concentration P_H	Osmotic pressure (atmospheres)	Specific resistance (ohms)	H ion concentration P_H
	Barley plants 8 weeks, grown in water cultures								
200 ...	0.07	3870	5.50	8.01	97	5.68	3.62	125	6.44
200 ...	0.07	3230	6.53	7.55	76	5.68	3.69	137	6.12
1400 ...	0.58	550	5.48	8.13	61	5.85	4.11	117	6.83
1300 ...	0.56	528	6.83	8.35	66	5.82	.....	.....	.....
2300 ...	0.88	347	5.14	8.86	65	5.99	5.04	102	6.97
2200 ...	0.88	347	6.76	10.26	50	5.90	4.52	119	7.08
4500 ...	1.72	196	4.94	11.40	53	5.95	4.90	95	.....
4300 ...	1.70	189	6.14	10.24	53	5.90	5.63	77	6.97
	Barley plants 7 weeks, grown in sand cultures								
2500 ...	0.94	335	6.97	10.66	46.9	6.12			
5000 ...	1.81	220	6.97	11.74	42.0	6.15			
8000 ...	2.75	148	6.97	12.24	38.9	6.20			
	Barley plants 6–7 weeks, grown in 6 different soils								
600 ...	0.22*	.....	7.03	9.96	59.9	0†			
600 ...	0.21	.....	7.15	9.52	60.4	6.12			
900 ...	0.33	.....	7.15	10.96	64.2	6.15			
500 ...	0.16	.....	7.03	10.14	69.2	0†			
500 ...	0.19	.....	7.17	9.97	71.5	5.99			
200 ...	0.08	.....	7.34	9.11	65.8	6.12			

* This column represents concentration of soil solution at about time plants were collected; previously the concentration was higher.

† No determination was made.

the osmotic pressure of the tops is much greater than that of the corresponding roots. These findings are in general agreement with those of McCOOL and MILLAR. The tops of the plants grown in the various soils have osmotic pressures similar to those found in the water culture experiments. The osmotic pressures of the soil solutions, as determined by the method of BOUYOUCOS and McCOOL, varied between 0.08 and 0.33 atmospheres at

about this stage of growth of the plant. They are thus quite comparable with the concentrations of certain of the solutions used in the sand and water culture experiments. In every instance the osmotic pressure of the plant sap is much higher than that of the nutrient medium.

All of the samples of plant sap have a very high specific conductivity, and very decided variations are exhibited by the different water cultures. The concentration of the nutrient solution has unquestionably had a pronounced effect on the electrolyte content of the cell sap. A somewhat greater resistance is found in the root sap than in that from the tops. It is to be noted, however, that the variations in conductivity due to the nutrient solution are generally as great for the tops as for the roots, but in either case only a general relationship is apparent. The conductivities of the plant saps from the various soils were very similar in magnitude to those of the plants grown in water culture solutions of 0.5 to 0.9 atmospheres osmotic pressure. The conductivities of the sand culture plants were somewhat greater, but these plants were two weeks younger. In each case the expressed cell sap has a much higher concentration of electrolytes than the surrounding media, the relationship being 50:1 in the case of the most dilute solution. Thus the nutrient media, the root sap, and sap from the tops show three very dissimilar levels of electrolyte concentration. That the simple laws of diffusion are not sufficient to explain the equilibria involved in the plant absorption and metabolism is well recognized, and the data now presented strikingly illustrate this point of view, with reference to the ion content of the plant and its nutrient solution.

H ion concentration of sap

HAAS (3) and TRUOG (7) have shown that the sap of plants quite generally has an H ion concentration distinctly on the acid side. Determinations of H ion concentration were made on the samples of sap obtained as previously described. Table I shows a comparison of the acidity of the sap from plants grown in water cultures of very different concentration and reactions, as well as in sand and soil media.

It is evident that all samples of tops have almost the same P_H value, although the electrical resistances and osmotic pressures may vary widely. It would seem that the reaction is governed by a definite buffer system. In this connection it will be of interest to state that other experiments reported elsewhere have shown that the plant possesses a marked regulatory influence in the selective absorption from the various phosphoric acid anions; that is, either an alkaline or acid nutrient solution has its reaction quickly changed to approximate neutrality.[1]

The measurements of H ion concentrations on the sap from the tops were very definite and constant, but the determinations on the root sap were less satisfactory. An increase in alkalinity was noted during the measurement, possibly due to the reduction of NO_3 and the absence of a sufficient buffer effect. Apparently, however, the sap expressed from the roots has a nearly neutral reaction in several cultures.

Chemical analyses of plant sap

The analyses presented in table II were made on the expressed sap from plants grown in 6 soils of different origin and productivity. The soils were kept at optimum moisture content and under strictly controlled conditions, and 6 or 7 weeks after planting one or two tillers were separated from each of about 20 plants for each soil examined. The sap was obtained by the procedure already described, and then diluted and filtered through a porcelain candle to separate out any suspended material. The analyses were made by the methods described by STEWART (6).

The content of the individual ions substantiates the high conductivity measurements on the sap. All ions are present in relatively great concentration, including the NO_3 ion. Some idea of the relation between the composition of the cell sap and soil solution may be gained by comparison with the data for the soil extracts made at about the same time. There is reason to believe that the relation of several important ions is somewhat

[1] Later experiments have indicated that the HCO_3 ion formed is of greatest importance in regulating the reaction of complete nutrient solutions. A solution of KH_2PO_4 alone retains about the same P_H value.

similar in the soil solution and soil extract. As previously shown, the extract may contain several times the quantity of solutes actually found in the soil solution; that is, in the free water of the soil when it contains optimum percentage of water. The magnitudes have been calculated by the methods of BOUYOUCOS and MCCOOL. Estimates of the concentration of ions present in the soil solution indicate that invariably the plant sap has a much greater concentration, and in the case of K and PO_4 ions many times greater. It is evident that the relation of the ions to each other is also quite different in the two cases. In the soil solution

TABLE II

ANALYSES OF PLANT SAP AND SOIL EXTRACTS

SOIL NO. AND TEXTURE	P.P.M. OF EXPRESSED SAP					P.P.M. OF 1:5 SOIL EXTRACT†				
	Total nitrogen*	Ca	K	Mg	PO_4	NO_3	Ca	K	Mg	PO_4
Silty clay loam 3.....	864	680	5540	300	840	90	70	50	20	9
Silty clay loam 5.....	1030	640	5660	280	1360	70	90	30	20	21
Fine sandy loam 8....	1370	920	6130	280	920	60	70	30	20	12
Fine sandy loam 9....	820	1040	5240	290	870	30	30	25	10	10
Fine sandy loam 11...	680	620	5690	280	1960	50	40	50	15	30
Fine sandy loam 12...	940	640	5440	340	1420	30	30	30	10	12

* From 30–50 per cent in form of NO_3 nitrogen.

† Extracts are much more dilute than soil solution, the total concentration of which is shown by table I.

Ca is present in about the same magnitude as K; in the plant sap the concentration of K is from 5 to 10 times that of Ca. The ratio of Ca to Mg is not very dissimilar, Ca exceeding Mg in all samples, both in the plant and soil.

The question has often been discussed whether the plant reflects the composition of the soil. It is the general consensus of opinion that the analysis of the plant ordinarily gives no indication of the deficiencies or fertilizer needs of a soil. This could scarcely be expected, of course, in view of the complex and changing nature of the two systems of the plant and soil. Moreover, analyses of the mature and ripened plant, such as usually have been made, could not possibly give any insight into the relation of the plant to the soil in the period of active growth. It cannot be too strongly emphasized that both the plant and soil are

dynamic systems. The soil solution, as previous work in this laboratory has definitely shown, is changing from day to day, and gradually attains a very low concentration at the time when the plant has completed its maximum absorption. Without consideration of these phenomena it would be useless to hope for any clear understanding of soil fertility problems as related to plant requirements.

That some relation must exist between the inorganic elements in the plant and its nutrient solution is apparent from the data obtained in the water culture experiments. Similarly, concentration and composition of the soil solution should affect the absorption by the plant, but here it is very difficult to establish the relationship. For this purpose it would be necessary to appraise not only the concentration of the soil solution at a given time, but its potentiality for renewal. In the work previously referred to an idea of this factor has been obtained by comparing cropped and uncropped soils. The use of this method has made it possible to show some rather definite relations between water extracts of the soils and crop yield, but in the experiment now under consideration the composition of the sap at the given period bears no constant relation to the final yield of the crop, nor to the composition of the water extracts. An exception to this statement may possibly be found in the case of phosphorus, where the concentration of PO_4 in the soil solution is not improbably reflected in the sap. The K and Mg, and to a less extent the Ca, are of approximately the same magnitudes in all samples. While the soils in question varied considerably in their productivity, apparently the average concentrations of the soil solutions were not sufficiently different clearly to influence the concentration of the cell sap.

It does not follow from the foregoing that further study will not indicate a connection between the soil solution and the elements absorbed by the plant. Before the question is decided it will be necessary to examine not only the cell sap, but the total composition and yield, with the strictest control of the soil and study, not of the ripened plant, but the plant in the various stages of active metabolism.

Summary

The expressed sap from barley plants grown in water, sand, and soil cultures under controlled conditions has been examined with the following results:

1. The osmotic pressures in the sand and water cultures are reflected in the cell sap of the tops and roots.

2. The electrical conductivity of the nutrient solution has a marked influence on the conductivity of the sap. This is as marked for the tops as for the roots. The conductivity of the plant sap is from 4 to 50 times greater than that of the nutrient solution.

3. The sap from the tops of all plants grown in sand and soil cultures or water cultures of different concentrations and reactions had almost the same P_H value, approximately 6.0.

4. Samples of sap from plants grown on 6 different soils under the same climatic conditions were analyzed for important elements. In every case the concentration in the sap was found to be very much greater than in the soil solution.

5. The dynamic nature of the relation between the soil solution and the plant is emphasized.

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BRIEFER ARTICLES

PARAFFIN SOLVENTS IN HISTOLOGICAL WORK

In the last few years there have been described in the BOTANICAL GAZETTE two ways of improving the common method of replacing xylol with paraffin in histological work. The alleged defect in the old method lies in the fact that the paraffin sinks to the bottom of the vessel and immediately surrounds the specimens with a concentrated solution. LAND'S[1] remedy for this is to support the paraffin near the surface of the xylol in a basket of wire gauze; GOODSPEED[2] molds the paraffin into a lump that will fit the containing vessel and rest on top of the xylol. Both of these methods have been found good for accomplishing the end desired, and a third may be of interest.

The paraffin to be used can, by aëration, be rendered capable of floating in xylol, the method of preparation being similar to that employed in giving buoyancy to some kinds of soap. While the paraffin is at a temperature only a degree or two above that required for melting, a current of cold air is bubbled through it, causing it to harden as a frothy mass. This mass is then kneaded to secure finer grain and more even distribution of the air bubbles. Only a small amount of air is needed to produce the proper buoyancy, and it can be supplied from any one of numerous sources and cooled by passing through a condenser. Although the method may seem troublesome, it is really not so; a large amount of paraffin may be prepared at one time, and nothing further is necessary to get the desired results.

Although this method of holding the paraffin at the top of the xylol has been found successful and easy of manipulation, I do not employ it as a rule, because I have not yet seen the defect of the old method of adding the paraffin when xylol is used, and chloroform has been found perfectly satisfactory in all cases. If, when xylol is used, the necessary amount of paraffin be added in a finely divided condition in 5 or 6 portions, and diffusion aided by gentle agitation, a

[1] LAND, W. J. G., Microchemical methods, an improved method of replacing the paraffin solvent with paraffin. BOT. GAZ. **59**:397. 1915.

[2] GOODSPEED, T. H., Method of replacing paraffin solvent with paraffin. BOT. GAZ. **66**:381–382. 1918.

sufficiently gradual change is secured. When the paraffin is to be supported at the surface of the solvent, by buoyancy or other means, it is better to add it in a round lump, because in this case slow diffusion is desirable; but, when it is allowed to sink in the xylol, it should be added in small fragments, since quick diffusion is desirable.

Although in many laboratories xylol has taken the place of chloroform in work of this kind, the latter is still used by many technicians because of certain advantages that it possesses over xylol. The object in using either is not, as is often stated, to "clear" the specimen, but to provide a medium for the introduction of the paraffin. Actual "clearing," that is, the production of transparency, is of no benefit at all in this stage of the process; in fact, it proves a disadvantage that can be overcome only by staining *in toto* when only a few sections from a long ribbon are to be selected for mounting. Chloroform has much less tendency to produce transparency than has xylol. Moreover, as DUGGAR[3] has stated, a peculiarly undesirable optical effect is produced permanently by xylol in some tissues. The use of chloroform makes unnecessary, of course, any device for holding the paraffin at the top, if this is thought necessary.

The higher specific density of chloroform, which LAND[4] has mentioned as a factor to be considered, is aside from the question; it has long been known that permeability and osmotic pressure, the things which determine shrinkage, are not, as was once supposed, determined by density when pure substances are being compared. Moreover, neither chloroform nor xylol could "plasmolyze" a cell, as LAND states; at this stage of the process a cell is not subject to such life phenomena as plasmolysis.

The item of expense, which has caused the use of chloroform to be discontinued in many places, and which is of special significance just now, can be lessened by the easy and inexpensive recovery of about one-half of the reagent used in the ordinary process. The waste chloroform, containing alcohol or other impurities, is thrown out of solution with a large quantity of water, dried with calcium chloride, which removes both alcohol and water, and distilled, the boiling point being observed as a check on the purity of the product.—PAUL WEATHERWAX, *Indiana University.*

[3] DUGGAR, B. M., Fungous diseases of plants. New York. 1909. p. 49.

[4] In a note added to an article by MOTTIER, BOT. GAZ. **61**: 253. 1916.

CURRENT LITERATURE

BOOK REVIEWS

Practical botany

A widespread belief that botany should be so taught and adapted that it should be of service in its industrial applications, particularly agriculture, has led to the publication of several texts with this in view. The production of such a text demands an author who is not only trained in botany, but also familiar with the practical bearings of the subject. The two books reviewed here were written to meet the real or supposed need indicated.

Cook[1] states that he has aimed at three things: "(1) a brief statement of the recognized facts and principles concerning plants and plant growth usually given in textbooks for secondary schools; (2) a list of simple exercises and suggestions for observations which the pupil can conduct without great difficulty and which will demonstrate many of the statements given in the book; (3) a list of questions which are intended to be suggestive to the pupils and to encourage further studies." "The title, 'Applied economic botany,' implies (1) that it is intended as a guide to experimental work in the study of plants, such as should be carried on in any high school, and (2) that it is intended as a preliminary work to the agricultural studies which are now recognized in many high schools."

The first part treats of seeds, roots, stems, buds, leaves, flowers, reproduction, fruits, anatomy, chemical composition, food, and growth, and also outlines the great groups. In short, it is in general the usual botanical foundation given in secondary schools. There are also chapters dealing with forestry, plant diseases, plant breeding, and weeds. Much space is given to "exercises" and "questions," since it is through these that the author aims to lead the student to nature for facts, holding that "we cannot, in the short time allowed to the subject, expect to learn much about many thousands of plants . . . . but we can learn certain principles of plant growth . . . . to be close observers . . . . to be accurate in our experiments and in making records." The exercises are so selected that the material is easily available, for they are based on plants familiar to all and usually of economic importance.

The second part treats of families, discussing the chief economic representatives of each, for example, celery, parsnip, carrot, etc., among the Umbelliferae, and coffee and quinine among the Rubiaceae. The general characters of

[1] Cook, M. T., Applied economic botany. 8vo. pp. xviii+261. *figs. 142.* Philadelphia: J. B. Lippincott. 1919.

the family are given concisely, as well as the more interesting features of the history, distribution, and uses of the more important economic representatives.

The book is written in attractive style, and the material is well selected, and is a commendable effort to differentiate secondary-school botany from university botany. The numerous half-tones are of unusually good quality.

MARTIN[2] has attempted the same task, except that his book is more specifically directed to the botanical needs of the student of agriculture. The first part deals with flowers, seeds, cells, roots, stems, buds, leaves, etc. The application to agriculture consists chiefly in the fact that economic plants are used as illustrative material. For example, oats, corn, wheat, pineapple, tomato, etc., are made to show the usual fundamental facts of morphology. The second part presents an outline of the plant kingdom, from Thallophytes to Angiosperms, along with chapters on ecology, evolution, heredity, and plant breeding.

The presentation throughout is botanical rather than agricultural, a foundation for agricultural study rather than a study of agriculture. The line drawings are not as well done or as accurate as they should be, and the illustrations in general are in contrast with the excellent presswork and the easy and pleasing style of presentation.—F. L. STEVENS.

NOTES FOR STUDENTS

Physiology of dormancy.—Recent work by CROCKER and HARRINGTON[3] materially increases our knowledge of the physiology of dormancy and germination of seeds, and throws much light on the problems of vitality and respiration. Differences in the optimum temperature requirements for germination of Johnson grass and Sudan grass led to a study of their physiological differences, and a comparison of their behavior with seeds of widely separated groups of plants. The discussion therefore is a general contribution of much significance to seed physiology. The study centers in the relation of catalase content to dormancy and vitality. Some improvements in methods of measuring catalase activity are suggested, chief of which is neutralization of the hydrogen peroxide used, as it is found to be injuriously acid if unneutralized. Degree of pulverization must be considered, as seeds vary somewhat as to optimum degree of fineness. Bolting cloth of 70–100 mesh gave the best results in the seeds used. Material must also be freshly ground, as degeneration of catalase is rapid after destruction of morphological integrity.

Catalase activity is found to be 28 or 29 times as great in the embryo of Stoner wheat as in the endosperm. Sudan grass and a hybrid between Tunis grass and sorghum gave similar results. Bracts surrounding the caryopses,

[2] MARTIN, J. N., Botany for agricultural students. 8vo. pp. xv+585. *figs. 488*. New York: John Wiley & Sons. 1919.

[3] CROCKER, WM., and HARRINGTON, G. T., Catalase and oxidase content of seeds in relation to their dormancy, age, vitality, and respiration. Jour Agric. Research **15**:137–174. 1918.

however, and sterile florets show very low activity, especially after a year of dry storage. It is suggested that in these cases, where presumably dead tissues give catalase reaction, the activity is a residuum of previous physiological activity. In the grasses, catalase activity decreases continuously from the time the seeds are harvested. This decrease, however, is in no way connected with loss of vitality. Even when the activity has fallen to one-half or one-third of the fresh harvested material, there may still be practically complete vitality, and but slight loss in vigor. The investigation indicates that under certain conditions catalase activity might be used to estimate the age of seeds. It would only be necessary to know the normal rate of activity decrease, to be sure that no accidental destruction of catalase activity had occurred, and to use proper controls of materials of known age and of equal maturity with the seed to be tested.

When dormancy occurs in grasses it is usually caused by seed coat characters. Thorough drying at about 20° C. is the best after-ripening condition, but during drying catalase activity is falling rapidly. Seeds of *Amaranthus retroflexus* also have a dry after-ripening period, but in these the catalase is time-stable, and very little decrease in catalase activity occurs during drying. In contrast to these there are other seeds, as peach, linden, and hawthorn, where dormancy is due to embryo conditions, in which after-ripening, which depends mostly on cool temperature and moisture, is accompanied by a rapid increase in catalase activity and other fundamental time-requiring chemical changes. Such chemical changes, aside from catalase decrease, do not occur in the grasses. Heating air-dry seeds finally reduces both vitality and catalase activity, but the denaturing of the catalase and the proteins upon which viability depends does not run parallel. Catalase decrease commences at once; but viability may increase temporarily, and then decrease to zero before the catalase is destroyed. In other cases viability is soon lost, but catalase activity remains high, as in *Amaranthus*.

Seeds of Johnson grass kept in a germinator at room temperature undergo secondary dormancy and lose their catalase activity rapidly. The respiratory activity falls correspondingly. If these conditions are repeated in seeds buried in the soil, they may have an important bearing on the longevity of buried seeds. If death depended upon destruction of food supply by respiration, decreased respiration would provide longevity. When seeds of Johnson grass germinate, there is a very rapid increase again in the catalase activity. At every point it seems that catalase activity and respiration intensity run parallel. Some tests of oxidase activity were made with the same seeds, and it is shown that oxidase activity decreases with age. Little relation was found between oxidase and after-ripening changes in grasses or peach seeds, and oxidase activity showed no increase in germination, but in those non-living parts, bracts and scales, where catalase activity was low, oxidase activity was relatively high. The oxidase of Johnson grass and the Tunis grass-sorghum hybrid is only slightly sensitive to mercuric chloride poisoning.

Another paper by the same authors[4] describes some experiments made to test the effects of desiccation on vitality of seeds. The seeds of grains and grasses will withstand drying to less than 1 per cent without material loss in germination. Blue grass and Johnson grass can even be dried to 0.1 per cent of moisture without loss in germination, but vigor is greatly reduced in the blue grass. Still further loss of vigor occurred in the blue grass when dried in vacuo at 100° C. for 6 hours, but the germination percentage was not materially reduced. These results negative the statements of EWART that excessive drying changes dormant protoplasm to such an extent that the essential molecular groupings cannot be re-established under conditions for germination.—C. A. SHULL.

Curing timber.—A method of drying timber more uniformly to avoid cracks and shakes in the logs is proposed by STONE.[5] The method is based upon assumptions as to the natural movement of sap in trees which will not meet with favor among plant physiologists. He considers that the water is held in the saturated tracheal walls, evaporates from these walls into continuous vapor-filled lumina, and moves upward through the tubes in response to a partial vacuum produced above by transpiration. Indeed, the water is supposed to travel upward mostly by night, because at that time the leaves are much cooler than the trunk, and would condense the vapor from the tubes, thus filling the cells as reservoirs against the next day's transpiration. Salts are imagined to travel through the cell walls of the tracheae rather than in the transpiration stream, which is nonexistent in STONE's assumption. It is hard to imagine a conception much more at variance with experimental results of physiological studies.

The actual drying plant suggested is a closed shed, arranged with a cooler at one end, the purpose of which is to condense the moisture as it leaves the logs, in the form of hoar frost, on the principle of the dew pond. Thus the air of the shed will be kept continually dry, and cold dry air constantly circulating through and around the porous logs. He asserts that this would dry each annual layer simultaneously, and that the shrinkage would be regular and occur without cracking. Whether the proposed plant would really result in the uniform curing of timber the reviewer must leave to the practical forester. Perhaps the suggestion is much sounder on the practical side than the assumptions on which it is based would seem to indicate.—C. A. SHULL.

Philippine plant diseases.—REINKING[6] has published an excellent and very useful account of the economic plant diseases of the Philippines, which

[4] HARRINGTON, G. T., and CROCKER, WM., Resistance of seeds to desiccation. Jour. Agric. Research 14:525–532. 1918.

[5] STONE, HERBERT, The ascent of the sap and the drying of timber. Quart. Jour. Forestry 12:261–266. 1918.

[6] REINKING, OTTO A., Philippine economic plant diseases. Philipp. Jour. Sci. 13:165–274. *pls. 20. figs. 43.* 1918.

will be welcomed by plant pathologists, especially those who are interested in tropical plant diseases. In the introduction the author states that the losses due to fungi are at least 10 per cent. He also states that "certain articles on phytopathology in the tropics give an entirely wrong impression of the number and destructiveness of the diseases." In the Malayan regions, at least so far as the Philippines are concerned, there are represented all groups of fungi that are present in the temperate regions. Extremely destructive diseases are produced by some of each group. It is very evident from this and other works that the diseases follow the host plants very closely. Agricultural plants, especially vegetables and truck crops that are very widely distributed, are attacked by the same pathogens, whether grown in the tropical or temperate zones. The coffee industry was at one time wiped out by a fungus, the cacao loss is about 50 per cent, and the rice losses are very heavy. The author lists 60 hosts of which about one-half are grown to a greater or less degree within the bounds of the United States, especially in the southern states or Pacific Coast states. There are a total of 339 diseases listed, many of which are found within the United States. The author gives brief but accurate descriptions of the symptoms, the causal organisms, and statements concerning the control measures. Ten pages are devoted to the discussion of spray mixtures and methods of control.—MEL T. COOK.

Root-nodules.—Miss SPRATT[7] has investigated the formation of root-nodules by *Bacillus radicicola*. The plants producing nodules when infected are sharply differentiated into 2 classes, legumes and non-legumes. In the Leguminosae the cortical cells respond to the stimulus, resulting in the nodule. In other plants the penetration of the bacteria into root-hairs and cortex induces no morphological change until a young lateral root is infected during its passage through the cortex, and as a consequence becomes swollen and forms the nodule. In other words, the root-tubercles of non-leguminous plants are modified lateral roots, while those of the legumes are exogenous in origin. A contrast in the structure of the 2 types of nodule is evident. In leguminous nodules the bacteroidal tissue is central, and the vascular system consists of a number of peripheral strands; while in the non-leguminous nodules the stele is central, retaining its connection with the root cylinder and growing point. In making a comparative study of the nodules of Leguminosae, Miss SPRATT recognizes 4 types, based chiefly upon the distribution of meristem, bacteroidal tissue, and vascular tissue, and these types are definite enough to characterize various groups of Leguminosae.

The author concludes that "the form of the nodule depends primarily on the nature of the environment of the host, which influences the cell-sap and consequently the behavior of the bacteria after they have entered, and secondarily on the anatomical peculiarities of the particular plant."—J. M. C.

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[7] SPRATT, ETHEL R., A comparative account of the root-nodules of the Leguminosae. Ann. Botany 33:189–199. *pl. 13. figs. 5.* 1919.

Soil fertility.—From a study of the distribution of phosphorus, nitrogen, calcium, potassium, and other elements in soils whose previous history and crop yields are known, certain Rothamsted plots, Pennsylvania State College fertility plots, and Strongsville, Ohio, plots, VAN ALSTINE[8] draws conclusions as to the movement of nutrient elements in the soil.

Phosphorus used as a fertilizer moves but slightly in the soil, remaining fixed until removed by plants, or by the erosive action of water or wind. Certain alkali salts stimulate a larger use of phosphorus, especially by legumes. Potassium also is readily held by the soil, but moves somewhat more freely than phosphorus when other fertilizer salts are used. It may then be carried down beyond the reach of the root system. Nitrogen leaches out into the drainage water to a certain extent, but the loss is small if the crop remains on the soil throughout the growing season. Nitrogen will even accumulate in the soil, mostly in roots and other residues. Carbonates wash out of the soil readily, particularly in the presence of ammonium salts. Magnesium also will leach under this circumstance. The calcium content of the soil decreases as the carbonates go out, and with alkali fertilizers it decreases more rapidly than acidity develops. In the presence of ammonium salts, calcium loss occurs just about as rapidly as acidity develops. Such facts as these should be taken into consideration in developing a rational fertilizer practice.—C. A. SHULL.

Suspensor of Trapa.—TISON[9] has described a remarkable suspensor developed by *Trapa natans*. For some time during embryogeny the suspensor region grows with remarkable vigor, the cells becoming numerous, as well as very large and turgid. The embryo is finally differentiated at the tip of the massive suspensor, which is also the source of nutritive supply to the embryo during its earlier stages. Similar suspensor behavior has been described by GUIGNARD among the Leguminosae, and by TREUB in certain Orchidaceae, but in none of them does the situation seem to be so extreme as in *Trapa*.—J. M. C.

Opuntia.—GRIFFITHS[10] has published 8 new species of *Opuntia*, which he has recognized in connection with cultures of *Opuntia* upon the Department of Agriculture grounds at Chico, California.—J. M. C.

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[8] VAN ALSTINE, E., The movement of plant food within the soil. Soil Science 6:281–308. 1918.

[9] TISON, M. A., Sur le suspenseur du *Trapa natans* L. Rev. Gén. Botanique 31:219–228. *pl. 4. figs. 5.* 1919.

[10] GRIFFITHS, DAVID, New and old species of *Opuntia*. Bull. Torr. Bot. Club 46:195–206. *pls. 9, 10.* 1919.